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To Eat or Not To Eat: Contributions of Dorsal Hippocampal Neurons and Memory to Meal Onset

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TO EAT OR NOT TO EAT: CONTRIBUTIONS OF DORSAL HIPPOCAMPAL NEURONS AND MEMORY TO MEAL ONSET

by

YOKO OGAWA HENDERSON

Under the Direction of Marise Parent, PhD

ABSTRACT

There is extensive research regarding the neural mechanisms that control satiety and meal termination; in contrast, there is very limited understanding of how the central nervous system regulates meal onset and thus the duration of the postprandial intermeal interval (ppIMI) and meal frequency. Based on emerging evidence, we hypothesize that dorsal hippocampal neurons, which are critical for episodic memory, form a memory of a meal and inhibit meal onset during the ppIMI. To test whether hippocampal neurons form a memory of a meal, we first determined that ingesting sucrose or isopreferred concentrations of the non-caloric sweetener saccharin increased the expression of the plasticity-related immediate early gene activity-

regulated cytoskeleton-associated protein (*Arc*) in dorsal CA1 hippocampal (dCA1) neurons in Sprague-Dawley rats. Furthermore, repeated exposure to the sucrose meal attenuated the ability of the sucrose to induce *Arc* expression. Together, these data indicate that orosensory stimulation produced by a sweet taste is sufficient to induce synaptic plasticity in dCA1 neurons in an experience-dependent manner. Second, we showed that reversibly inactivating dorsal hippocampal neurons with infusions of the $GABA_A$ agonist muscimol after the end of a sucrose meal accelerated the onset of the next meal, indicating that dorsal hippocampal neurons inhibit meal onset. Lastly, using a clinically-relevant animal model of early life inflammatory injury, we found that neonatal injury (1) impairs hippocampal-dependent memory, (2) decreases the ppIMI and increases sucrose intake, (3) increases body mass, (4) attenuates sucrose-induced *Arc* expression in dCA1 neurons, and that (5) blocking inflammatory pain with morphine at the time of injury reverses the effects of injury on memory, energy intake and *Arc* expression. Collectively, the findings of this dissertation support the overarching hypothesis that dorsal hippocampal neurons inhibit meal onset during the ppIMI and suggest that dorsal hippocampal dysfunction may contribute to the development and/or maintenance of diet-induced obesity.

INDEX WORDS: Sucrose, Saccharin, Memory, Activity-regulated cytoskeleton-associated protein, Muscimol, Morphine

TO EAT OR NOT TO EAT: CONTRIBUTIONS OF DORSAL HIPPOCAMPAL NEURONS AND MEMORY TO MEAL ONSET

by

YOKO OGAWA HENDERSON

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DEDICATION

I am dedicating this dissertation to my best friend and my husband **Michael J. Henderson**. I complained, whined, cried, and wanted to give up so many times, but you made me believe that everything is possible. I could not have done this without you. Thank you!

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LIST OF ABBREVIATIONS

ANOVA, analysis of variance

AMPAR, α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor

Arc, activity-regulated cytoskeleton-associated protein

ARC, arcuate nucleus of the hypothalamus

BBB, blood-brain barrier

BDNF, brain derived neurotrophic factor

CGN, carrageenan

CeA, central amygdala

CORT, corticosterone

dCA1, dorsal hippocampus CA1 subfield

Ctrl, caged controls

FISH, fluorescent *in situ* hybridization

FST, forced swim test

GR, glucocorticoid receptor

HC, hippocampus

HPA, hypothalamic-pituitary-adrenal

IL-1β, interleukin 1β

IEG, immediate early gene

LH, lateral hypothalamus

LTP, long-term potentiation

mCVS, mild chronic variable stress

MUS, muscimol

NAc, nucleus accumbens

NICU, neonatal intensive care unit

P, postnatal day

ppIMI, postprandial intermeal interval

pCREB, phosphorylated cAMP response element-binding protein

ROI, region of interest

Sacch, saccharin

Suc, sucrose

TNFα, tumor necrosis factor alpha

US, United States

Veh, vehicle

1 CHAPTER 1: GENERAL INTRODUCTION

1.1 Background

A wide variety of species, including both vertebrates and invertebrates, consume food in bouts (i.e., meals; Simpson & Bernays, 1983; G. P. Smith, 2000). There is a wealth of knowledge regarding the mechanisms that determine when to stop eating (Clifton, 2000; Davis, Smith, Singh, & McCann, 2001; G. P. Smith, 1996, 2000, 2001; Strubbe & Woods, 2004); in contrast, there is a very limited understanding of the mechanisms that control meal onset and the duration of the postprandial intermeal interval (ppIMI). The ppIMI is the time spanning from the end of one meal to the beginning of the next meal; therefore, the duration of the ppIMI influences meal frequency and thus impacts total intake.

To date, evidence suggests that internal signals generated by a previous meal, environmental stimuli, and conditioned drives interact to influence meal onset and the duration of the ppIMI (Collier, Johnson, & Mitchell, 1999; Cornell, Rodin, & Weingarten, 1989; Kraly, Carty, & Smith, 1978; Petrovich, Ross, Holland, & Gallagher, 2007; Reppucci & Petrovich, 2012; Sclafani & Rendel, 1978; Strubbe, Keyser, Dijkstra, & Prins, 1986; Strubbe & van Dijk, 2002; Weingarten, 1983, 1984). What little is known is that internal hunger signals, endogenous biological clocks, and environmental and social cues associated with palatable food are involved. For example, decreases in plasma glucose concentrations can stimulate eating (Campfield & Smith, 2003). Lesions of the suprachiasmatic nucleus, which controls circadian rhythms, disrupt meal timing (Kersten, Strubbe, & Spiteri, 1980; Le Magnen, 1969; Van den Pol & Powley, 1979).

Furthermore, the presentation of food or cues associated with palatable food will cause a sated rat or human to eat, although it is not clear whether these reflect effects on the duration of the ppIMI (Birch, McPhee, Sullivan, & Johnson, 1989; Cornell et al., 1989; Sclafani & Rendel, 1978; Weingarten, 1983).

We hypothesize that top-down cognitive processes, such as hippocampal-dependent memory, control meal onset and thus meal frequency (Parent, Darling, & Henderson, 2014). Evidence is emerging that the dorsal hippocampus, which is a brain region important for episodic (autobiographical) memory (Barbosa, Pontes, Ribeiro, Ribeiro, & Silva, 2012; Hoge & Kesner, 2007; Kesner, Hunsaker, & Warthen, 2008; Li & Chao, 2008; Manns, Howard, & Eichenbaum, 2007; Quinn, Wied, Ma, Tinsley, & Fanselow, 2008), is also involved in the control of eating and energy regulation (Benoit, Davis, & Davidson, 2010; Davidson et al., 2010; Davidson, Kanoski, Schier, Clegg, & Benoit, 2007; Davidson, Kanoski, Walls, & Jarrard, 2005; Kanoski & Davidson, 2011; Tracy, Jarrard, & Davidson, 2001). For example, disrupting the encoding of an autobiographical memory of a meal (e.g., by watching television) in human participants increases the amount that is later consumed (Higgs & Donohoe, 2011; Higgs & Woodward, 2009; Mittal, Stevenson, Oaten, & Miller, 2011; Oldham-Cooper, Hardman, Nicoll, Rogers, & Brunstrom, 2011; Robinson et al., 2013); whereas recalling and enhancing the memory of a recently consumed meal decreases the amount that is subsequently ingested (Higgs, 2002; Higgs & Donohoe, 2011; Robinson et al., 2013).

Hippocampal neurons receive neural signals regarding food stimuli from multiple brain regions that are important for food intake, including the arcuate nucleus, the nucleus of the solitary tract, insula, and the orbitofrontal cortex (Amaral, Insausti, &

Cowan, 1987; Insausti, Amaral, & Cowan, 1987; Rolls, 2008; G. P. Smith, 2000; Wang et al., 2008; Wang et al., 2006). Hippocampal neurons also send extensive efferent projections to brain areas involved in energy regulation, including the hypothalamus (Cenquizca & Swanson, 2006), nucleus accumbens (Brog, Salyapongse, Deutch, & Zahm, 1993; Groenewegen, Vermeulen-Van der Zee, te Kortschot, & Witter, 1987) and lateral septum (Cenquizca & Swanson, 2006). Moreover, the hippocampus contains a multitude of receptors for pre- and postprandial hormones and adiposity signals, such as bombesin, ghrelin and leptin (Lathe, 2001). Interestingly, manipulating the activity of these hormones in the hippocampus influences non food-related memory (Harvey, Solovyova, & Irving, 2006; Matsushita et al., 2003; Zhao, Chen, Quon, & Alkon, 2004). In addition, leptin infusions to the ventral hippocampus reduce the expression of a conditioned place preference for a context previously associated with food (Kanoski et al., 2011), and manipulations that impair hippocampal functioning increase food intake and disrupt the ability of rats and humans to use interoceptive cues to guide behavior (Davidson et al., 2009; Davidson & Jarrard, 1993; Davidson et al., 2010; Higgs, 2008; Higgs, Williamson, Rotshtein, & Humphreys, 2008; Hock & Bunsey, 1998).

There is a small body of evidence that implicates hippocampal neurons in meal *onset* in particular. For example, the famous patient H.M. and other humans with hippocampal-dependent memory deficits do not perceive hunger and satiety normally, do not remember eating, and will eat an additional meal when presented with food, even if they have just eaten to satiety (Hebben, Corkin, Eichenbaum, & Shedlack, 1985; Higgs et al., 2008; Rozin, Dow, Moscovitch, & Rajaram, 1998). Furthermore, rats with transection of the fornix or excitotoxic hippocampal lesions eat small meals more

frequently than do control rats (Clifton, Vickers, & Somerville, 1998; Davidson & Jarrard, 1993; Osborne & Dodek, 1986).

Based on the evidence reviewed above, **our overarching hypothesis is that dorsal hippocampal neurons form a memory of a meal and temporarily inhibit meal initiation during the ppIMI (Figure 1.1).** This hypothesis will be addressed by the following specific aims.

1.2 Specific Aim 1: Do dorsal hippocampal neurons form a memory of an eating episode? (Henderson, Nalloor, Vazdarjanova, & Parent, in preparation)

Experiment 1 tested the hypothesis that dorsal hippocampal neurons form a memory of a meal. If the dorsal hippocampus forms a memory of a meal, then consuming a meal should induce synaptic plasticity in dorsal hippocampal neurons. We tested this by using a highly sensitive fluorescent *in situ* hybridization (FISH) technique to measure intra-nuclear activity-regulated cytoskeleton-associated protein (*Arc*) mRNA fociin the CA1 subfield of dorsal hippocampal (dCA1) neurons following a sucrose meal (Guzowski, McNaughton, Barnes, & Worley, 2001; Vazdarjanova, McNaughton, Barnes, Worley, & Guzowski, 2002). We measured *Arc* rather than other immediate early genes (IEGs; e.g., fos or zif/268) because *Arc* expression reflects plasticity rather than neuronal activity. For example, manipulations that impair *Arc* in the hippocampus produce memory deficits (Guzowski et al., 2000; McIntyre et al., 2005; Messaoudi et al., 2007; Plath et al., 2006) and importantly, the amount of learning-induced *Arc* expression is correlated with electrophysiological measures of plasticity rather than with rate of neuronal firing (Carpenter-Hyland, Plummer, Vazdarjanova, & Blake, 2010). Additionally, we focused our efforts on mRNA rather than protein because intra-nuclear

Arc mRNA is more temporally related to the inducing event (i.e., the meal) than protein, and *Arc* mRNA is translated into protein with high fidelity (Ramirez-Amaya et al., 2005). We measured *Arc* in dCA1 neurons following a sucrose meal because this subfield is critical for episodic memory (Barbosa et al., 2012; Farovik, Dupont, & Eichenbaum, 2010; Hunsaker, Lee, & Kesner, 2008).

Experiment 2 tested whether increasing the amount of previous experience with the sucrose solution, which would decrease the mnemonic demands associated with that meal, would also attenuate sucrose-induced *Arc* expression. Furthermore, Experiment 3 tested the hypothesis that orosensory stimulation is sufficient to induce *Arc* expression by comparing the effects of consuming sucrose, which produces both orosensory stimulation and postingestive consequences, to consuming saccharin, which produces similar orosensory stimulation but no significant postingestive effects (Byard & Golberg, 1973; Mook, Bryner, Rainey, & Wall, 1980; Renwick, 1985, 1986; Sclafani & Nissenbaum, 1985). If dorsal hippocampal neurons form a memory of an eating episode, then consumption of either a sucrose solution or a saccharin solution should increase *Arc* mRNA expression in dCA1 neurons.

1.3 Specific Aim 2: Do dorsal hippocampal neurons inhibit meal onset? (Henderson, Smith & Parent, *Hippocampus*, 2013)

Experiment 4 tested the hypothesis that dorsal hippocampal activity during the postprandial period is necessary to inhibit onset of the next meal. If dorsal hippocampal neurons inhibit meal onset, then temporary inactivation of dorsal hippocampal neurons timed to occur during the postprandial period when the memory of a meal is being formed and stabilized (i.e., consolidated) should accelerate onset of the next meal.

1.4 Specific Aim 3: Does neonatal inflammatory pain impair hippocampal-dependent memory? (Henderson, Victoria, Inoue, Murphy, & Parent, *Neurobiology of Learning and Memory*, in press)

If hippocampal-dependent memory controls meal onset, then pain-induced chronic hippocampal-dependent memory impairments should increase intake and body mass. To test this prediction, we proposed to use an animal model of early life inflammatory pain for chronic hippocampal-dependent memory deficits.

In the United States alone, approximately 500,000 babies are born prior to 37 weeks gestation and are considered preterm (J. A. Martin, Hamilton, Osterman, Curtin, & Mathews, 2013; Statistics, 2014). Premature infants spend an average of 25 days in the neonatal intensive care unit (NICU), where they undergo 10-18 invasive and painful procedures each day, including endotracheal intubation, surgery, catheterization, and mechanical ventilation (Barker & Rutter, 1995; Carbajal et al., 2008; March of Dimes Perinatal Data Center, National Perinatal Information System, & Services, 2011; Simons et al., 2003). Although preterm infants can respond to painful stimuli (Anand & Hickey, 1987; Bartocci, Bergqvist, Lagercrantz, & Anand, 2006; Grunau et al., 2005; Slater et al., 2006), approximately 65% of these procedures are performed in the absence of analgesia (Bouza, 2009; Carbajal et al., 2008; Rodkey & Pillai Riddell, 2013; Simons et al., 2003; Walter-Nicolet, Annequin, Biran, Mitanchez, & Tourniaire, 2010).

Evidence suggests that neonatal pain activates the hypothalamic-pituitary-adrenal (HPA) axis. In preterm infants, the number of skin-breaking procedures in the NICU is associated with increased cortisol levels in later development (8-18 months; Grunau et al., 2007; Grunau, Weinberg, & Whitfield, 2004). A preclinical study on the

effects of early life pain in rodents also found significantly elevated corticosterone (CORT) levels 24 h following inflammatory pain, with CORT levels remaining elevated above handled controls at 7 days post-injury (Victoria, Karom, Eichenbaum, & Murphy, 2014).

Stress and associated high levels of CORT negatively impact the hippocampus. Increased CORT down-regulates hippocampal glucocorticoid receptor (GR) expression (Kitraki, Kremmyda, Youlatos, Alexis, & Kittas, 2004), decreases dendrite number (Conrad & Bimonte-Nelson, 2010; McLaughlin, Gomez, Baran, & Conrad, 2007) and synapse density (Tata, Marciano, & Anderson, 2006), and impairs hippocampal-dependent memory (Conrad, Lupien, & McEwen, 1999; Conrad, Lupien, Thanasoulis, & McEwen, 1997; McLaughlin et al., 2007; Wright & Conrad, 2005). Similarly, neonatal pain in rats produces a decrease in GR mRNA and protein in adult dCA1 (Victoria, Inoue, Young, & Murphy, 2013a; Victoria, Karom, Eichenbaum, et al., 2014). Because increased CORT and decreased hippocampal GR expression are associated with significant memory deficits, these data suggest collectively that the stress associated with unresolved neonatal pain may produce long-lasting deficits in hippocampal-dependent function. Therefore, Experiment 5 tested the hypothesis that neonatal inflammatory pain produces long-lasting hippocampal-dependent memory deficits. In addition, Experiment 6 tested whether chronic stress, which produces a negative impact on hippocampal function (Diamond, Fleshner, Ingersoll, & Rose, 1996; Kleen, Sitomer, Killeen, & Conrad, 2006; Luine, Villegas, Martinez, & McEwen, 1994; Park, Campbell, & Diamond, 2001), exacerbates the effects of neonatal pain on memory. Furthermore, we tested whether neonatal inflammatory pain is necessary to produce memory deficits by

determining whether preemptive morphine treatments given at the time of injury would prevent the effects of injury on memory.

1.5 Specific Aim 4: Are pain-induced chronic hippocampal-dependent memory deficits associated with a shorter ppIMI and an increased body mass? (Henderson, Nalloor, Vazdarjanova, Murphy, & Parent, in preparation)

If hippocampal-dependent memory inhibits meal onset, then pain-induced chronic hippocampal-dependent memory impairments should be associated with a shorter ppIMI and increased total intake, which would be expected to increase body mass over time. Thus, Experiment 6 tested the hypothesis that neonatal inflammatory pain accelerates meal onset and increases total intake. The ability of morphine to attenuate the long-term consequences of early life pain on sucrose intake was also examined in Experiment 6. Experiment 7 tested the hypothesis that neonatal inflammatory pain increases body mass over time.

1.6 Specific Aim 5: Are pain-induced chronic hippocampal-dependent memory deficits associated with attenuated eating-induced *Arc* expression in dorsal hippocampal neurons? (Henderson, Nalloor, Vazdarjanova, Murphy, & Parent, in preparation).

If dorsal hippocampal neurons form a memory of an eating episode, then a manipulation that produces hippocampal-dependent memory deficits should also attenuate eating-induced *Arc* in dCA1 neurons. Thus, Experiment 8 tested the hypothesis that neonatal inflammatory pain attenuates eating-induced *Arc* expression in dCA1. We further tested the hypothesis that neonatal inflammatory pain is necessary by determining whether preemptive morphine treatments given at the time of injury would

prevent the effects of injury on eating-induced *Arc* expression.

1.7 Significance

Obesity is a major public health problem in the United States US. Approximately 35% of the current adult population in the US is obese (Ogden, Carroll, Kit, & Flegal, 2014). Overweight and obesity are associated with the development of potential deadly diseases, such as cardiovascular disease, type 2 diabetes, and coronary heart disease (Curb & Marcus, 1991; Hubert, Feinleib, McNamara, & Castelli, 1983; Must et al., 1999). The fact that the prevalence of obesity remains high demonstrates that current methods to prevent and treat obesity are not effective. We propose a novel hypothesis that suggests that hippocampal-dependent episodic memory deficits contribute to the development and maintenance of diet-induced obesity (Parent et al., 2014). Specifically, we hypothesize that impaired hippocampal function accelerates meal onset, leading to an increase in energy intake, eventually resulting in the onset of obesity (Parent et al., 2014). This dissertation used behavioral, pharmacological, and biochemical techniques in nine experiments to test this hypothesis.

1.8 Chapter 1 Figure

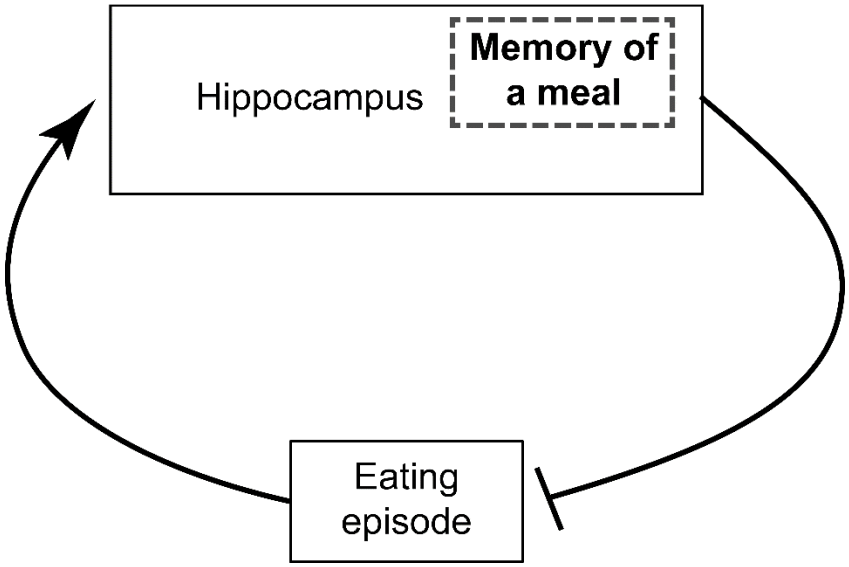

Figure 1.1 Proposed neural regulation of meal onset

2 CHAPTER 2: SWEET OROSENSATION INDUCES ARC EXPRESSION IN DORSAL HIPPOCAMPAL CA1 NEURONS IN AN EXPERIENCE-DEPENDENT MANNER

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2.1 Abstract

Decades of research suggest that different mechanisms regulate meal *initiation* (when to start eating) versus meal *termination* (how much to eat in a meal; satiety). Compared to our understanding of the factors that influence satiety, there is relatively little knowledge of the neural controls of meal onset and the duration of the postprandial intermeal interval (ppIMI). We hypothesize that dorsal hippocampal neurons, which are critical for episodic memory, form a memory of a meal and inhibit meal onset during the postprandial period. If dorsal hippocampal neurons form a memory of a meal, then consuming a meal should induce synaptic plasticity in dorsal hippocampal neurons. To test this, we used fluorescence *in situ* hybridization procedures to determine (1) whether

ingesting a sucrose meal increases expression of the plasticity-related immediate early gene activity-regulated cytoskeleton-associated protein (*Arc*) in dorsal CA1 hippocampal (dCA1) neurons, (2) whether increasing the amount of previous experience with the sucrose solution would attenuate sucrose-induced *Arc* expression, and (3) whether the orosensory stimulation produced by the noncaloric sweetener saccharin would be sufficient to induce *Arc* expression. Male Sprague-Dawley rats were trained to consume a sweetened solution at a scheduled time daily and on the experimental day were given a solution for 7 min and then euthanized immediately. Consuming sucrose (4 or 32%) or saccharin (0.2%) increased *Arc* in dCA1 neurons compared to cage control rats; moreover, the amount of sucrose-induced *Arc* expression was inversely correlated with the degree of previous experience with sucrose. The present study is the first to demonstrate that orosensory stimulation produced by consuming a sweetened solution and possibly the hedonic value of that sweet stimulation induces synaptic plasticity in dCA1 neurons. Collectively, these findings are consistent with our hypothesis that dorsal hippocampal neurons form a memory of a meal.

2.2 Introduction

Decades of research suggest that different mechanisms regulate meal *initiation* (when to start eating) versus meal *termination* (how much to eat in a meal; satiety). Compared to our understanding of the factors that influence satiety, there is relatively very limited knowledge of the neural controls of meal onset and the duration of the postprandial intermeal interval (ppIMI), defined as the time spanning from the end of one meal to the beginning of the next meal. The duration of the ppIMI influences meal

frequency and thus impacts total intake. To date, evidence suggests that internal signals generated by a previous meal, environmental stimuli, and conditioned drives interact to influence meal onset and the duration of the ppIMI (Collier et al., 1999; Cornell et al., 1989; Kraly et al., 1978; Petrovich et al., 2007; Reppucci & Petrovich, 2012; Sclafani & Rendel, 1978; Strubbe et al., 1986; Strubbe & van Dijk, 2002; Weingarten, 1983, 1984).

We hypothesize that cognitive factors, such as hippocampal-dependent memory, also control meal onset and meal frequency. Specifically, we hypothesize that dorsal hippocampal neurons, which are critical for episodic (autobiographical) memory (Barbosa et al., 2012; Hoge & Kesner, 2007; Kesner et al., 2008; Li & Chao, 2008; Manns et al., 2007; Quinn et al., 2008) form a memory of a meal and temporarily inhibit meal initiation during the ppIMI. In support of this, we recently demonstrated that temporary inactivation of dorsal hippocampal neurons timed to occur during the postprandial period when the memory of a meal was being formed and stabilized (i.e, consolidated) affected the timing of the next meal (Henderson, Smith, & Parent, 2013). More specifically, dorsal hippocampal infusions of the $GABA_A$ agonist muscimol given after rats had finished a sucrose meal accelerated the onset of the next meal and increased the amount consumed in the next meal. Our results are consistent with previous findings showing that posttraining reversible inactivation of dorsal hippocampal neurons impairs consolidation of other types of memory: place avoidance (Cimadevilla, Wesierska, Fenton, & Bures, 2001; Lorenzini, Baldi, Bucherelli, Sacchetti, & Tassoni, 1996), object-place recognition (Oliveira, Hawk, Abel, & Havekes, 2010), and spatial water maze (Cimadevilla, Miranda, Lopez, & Arias, 2008; Holahan & Routtenberg,

2011). In human participants, disrupting the encoding of the memory of a meal (e.g., by watching television) increases the amount that is consumed at the next eating bout (Higgs & Donohoe, 2011; Higgs & Woodward, 2009; Mittal et al., 2011; Oldham-Cooper et al., 2011; Robinson et al., 2013); whereas, recalling and enhancing the memory of a recently consumed meal decreases the amount that is subsequently ingested (Higgs, 2002; Higgs & Donohoe, 2011; Robinson et al., 2013). Furthermore, the famous patient H.M. and other patients suffering from hippocampal-dependent memory deficits do not perceive satiety normally, do not remember eating, and will eat an additional meal when presented with food, even if they have just eaten to satiety (Hebben et al., 1985; Higgs et al., 2008; Rozin et al., 1998).

If dorsal hippocampal neurons form a memory of a meal then consuming a meal should induce synaptic plasticity in dorsal hippocampal neurons. We tested this in the present experiment by using a highly sensitive fluorescent *in situ* hybridization (FISH) technique to measure activity-regulated cytoskeleton-associated protein (*Arc*) mRNA nuclear foci in the CA1 field of dorsal hippocampal neurons (dCA1) following a sucrose meal (Guzowski, McNaughton, et al., 2001; Vazdarjanova et al., 2002). We focused on dCA1 because this subfield is critical for episodic memory (Barbosa et al., 2012; Farovik et al., 2010; Hunsaker et al., 2008). We measured *Arc* rather than other IEGs (e.g., fos or zif/268) because *Arc* expression reflects plasticity rather than neuronal activity. For example, manipulations that impair *Arc* in the hippocampus produce memory deficits (Guzowski et al., 2000; McIntyre et al., 2005; Messaoudi et al., 2007; Plath et al., 2006) and importantly, the amount of learning-induced *Arc* expression is correlated with electrophysiological measures of plasticity rather than with rate of neuronal firing

(Carpenter-Hyland et al., 2010). We focused our efforts on mRNA rather than protein because intra-nuclear *Arc* mRNA is more temporally related to the inducing event (i.e., the meal) than protein and *Arc* mRNA is translated into protein with high fidelity (Ramirez-Amaya et al., 2005). Thus, measuring the number of *Arc* mRNA-expressing neurons in the dorsal hippocampus following a meal is a powerful strategy for detecting synaptic plasticity associated with eating a meal. We further tested the hypothesis that hippocampal neurons form a memory of a meal by assessing whether increasing the amount of previous experience with the sucrose solution, which would decrease the mnemonic demands associated with that meal, would also attenuate sucrose-induced *Arc* expression. Furthermore, we determined whether orosensory stimulation is sufficient to induce *Arc* expression by comparing the effects of consuming sucrose, which produces both orosensory stimulation and postingestive consequences, to consuming saccharin, which produces similar orosensory stimulation but no significant postingestive effects (Byard & Golberg, 1973; Mook et al., 1980; Renwick, 1985, 1986; Sclafani & Nissenbaum, 1985).

2.3 Materials and Methods

2.3.1 Animals

Adult male Sprague-Dawley rats (postnatal day [P] 54 upon arrival; Charles River Laboratories, Inc., Wilmington, MA) were housed individually in Optirat® cages (Animal Care Systems, Inc., Centennial, CO) under a 12:12-h light: dark cycle with free access to pelleted rat chow and tap water (unless otherwise stated). The rats were allowed to acclimate for at least 5 days after their arrival before any procedures were performed.

The Georgia State University Institutional Animal Care and Use Committee approved all animal procedures.

2.3.2 Behavioral Procedures

Experiment 1 determined whether sucrose consumption would increase *Arc* expression in dCA1 neurons (Figure 2.1). Rats (n = 6) were trained to consume a 32% sucrose solution at a scheduled time in the same location daily in order to minimize the contributions of novelty, spatial or contextual processes. We used the sucrose solution as the meal because (1) it is very palatable/rewarding to rats (Hajnal, Smith, & Norgren, 2004; G. P. Smith, 2004), (2) its stimulus qualities are more specific than meals that include fats and proteins, (3) many of its peripheral and central processing sites and mechanisms have been identified (Levine, Kotz, & Gosnell, 2003; G. P. Smith, 2004), (4) its concentration can be varied to manipulate postingestive consequences (Davis, Smith, & Singh, 2000; Kirkham & Cooper, 1988; Waldbillig & Bartness, 1982), (5) its effects can be compared to those of non-caloric sweeteners such as saccharin, and (6) it cannot be hoarded.

On the first training day, the rats were removed from their home cages at lights-on, placed into polycarbonate experimental cages with ALPHA-dri® bedding (Shepard Specialty Papers, Richland, MI) that did not contain food or water, and transported to the behavioral testing room. After 8 h they were presented with a bottle containing a 32% sucrose solution for 30 min. One hour later, they were returned to their home cages where chow and tap water were available *ad libitum* until the following day. This constituted one training trial and the rats were trained in the same manner for the next 4 days. Rats do not lose any weight on this regimen, presumably because they make up

for any deficit when provided with food and water *ad libitum* in the *vivarium*. Caged control rats (n = 2) were also placed into experimental cages and brought to the behavioral testing room, but food and chow were not removed and they were not given the sucrose solution. Starting on the 6th training day, the rats were trained in the same manner with the exception that the sucrose was given after 3 h rather than after 8 h. We started with an 8-h deprivation period in order to increase the likelihood that the rats would approach the bottle, but then decreased it to 3 h in order to be within the range of an average ppIMI (Snowdon, 1969). Rats were trained daily for a total of 10 days (5 days 8-h fast, then 5 days 3-h fast).

The experimental day occurred at least 24 h after the last training day. The rats were placed in the experimental cages that did not contain food at lights-on and brought to the behavioral testing suite. After 3 h, a bottle containing the 32% sucrose solution was attached to the front of their cage. After 7 min, the rats were removed from the experimental cages and anesthetized in a plastic gas induction chamber with 5% isoflurane gas (Baxter International, Deerfield, IL) in 1000mL/min of oxygen (Airgas, Inc., Radnor, PA) until they lost their righting reflex (< 1 min). They were then decapitated using a guillotine and their brains were harvested rapidly, flash frozen in chilled 2-methylbutane (Thermo Fisher Scientific, Inc., Waltham MA), and then stored at -80 ˚C. The meal was terminated after 7 min in order to maximize the ability to detect intra-nuclear *Arc* foci that were specifically activated from consuming the solution (Vazdarjanova et al., 2002). Caged control rats were fasted for 3 h and 7 min, after which they were euthanized and their brains processed in the same manner.

Experiment 2 determined whether increasing the amount of previous experience with the sucrose solution, which would be expected to decrease the mnemonic demands associated with that meal, would also attenuate sucrose-induced Arc expression. The same training procedures were used as in Experiment 1, with the exception that the rats were given access to water starting on the second training day in order to avoid the contributions of thirst. To manipulate amount of experience with the solution prior to the experimental day, one group of rats (n = 7) was trained until their latencies to lick from the bottle were less than 60 s for 2 consecutive days, and a second group was trained with a more stringent criterion (less than 30 s for 2 consecutive days; n = 6). Rats were assigned to the 30 s or 60 s group based on their latencies to euthanize a 30- and 60 s rat on the same day. That is, the 30- and 60 s groups were matched on number of training trials (7.83 ±1.56 training trials in the 30- s group and 6.43 ±1.13 in the 60 s group). Five cage control rats were included.

Experiment 3 determined whether orosensory stimulation would be sufficient to increase *Arc* expression in dCA1. Rats were randomly assigned to a 4% sucrose solution group (n = 3), a 0.2% saccharin solution group (n = 4), or a caged control group (n = 3). The 4% sucrose and the 0.2% saccharin solutions were used because they are isopreferred by rats (Messier & White, 1984; J. C. Smith & Sclafani, 2002), and saccharin provides orosensory stimulation similar to sucrose without any significant postingestive effects (Byard & Golberg, 1973; Mook et al., 1980; Renwick, 1985, 1986; Sclafani & Nissenbaum, 1985). The rats were trained to consume the assigned solution for 5 days consecutively as in Experiment 2. On the 6th training day, their assigned sweetened-solution was switched to the other solution, and they were then trained for

an additional 5 days. The order of the solutions was counterbalanced across rats. We elected to train the rats for a fixed amount of days rather than using a training criterion in order to provide equal exposure to both solutions. The bottles were weighed before and after each training trial and a sucrose/saccharin preference ratio (mean sucrose consumption [g]/mean saccharin consumption [g]) was computed.

2.3.3 Fluorescence in situ Hybridization (FISH)

Right hemispheres of brains were blocked in freezing media and 20 µm coronal sections were obtained using a cryostat, mounted onto glass slides, and stored at -20 ℃ until FISH procedures were performed. The slides were processed for FISH in the following manner: After fixing the tissue in 4% paraformaldehyde and permeabilizing in a 1:1 solution of acetone and methanol, a fluorescein-labeled full-length digoxigenin-labeled *Arc* antisense riboprobe was applied and hybridized overnight at 56 ℃. Following quenching of peroxidase activity, the digoxigenin tag was revealed with peroxidase-conjugated anti-digoxigenin antibody (Roche, Indianapolis, IN) and a tyramide amplification reaction using SuperGlo™ fluorescein (Green Fluorescent Solutions, Augusta, GA). The riboprobes were generated using MAXIscript® (Ambion, Austin, TX) *in vitro* transcription kits and digoxigenin-labeled UTP (Roche, Indianapolis, IN). Nuclei were counterstained with DAPI.

2.3.4 Image Acquisition and Stereological Analysis

Image stacks from dCA1 (2.8–3.8 mm posterior to bregma) were collected from at least three different slides from each animal using a 20x objective on a Zeiss AxioImager/Apotome system (Carl Zeiss, Dublin, CA). Unbiased stereological cell counting and classification were performed as follows: (1) neuron-like cells in dCA1 in

each image were segmented using an optical dissector method (West, 1999), and (2) segmented neurons were classified using Zeiss AxioVision imaging software (Carl Zeiss, Dublin, CA). Putative glial cells, which are those with small, intensely, and uniformly stained nuclei, were excluded from the analysis. Cells were classified as *Arc* positive if they contained foci of transcription for *Arc*. Cells without any foci were classified as *Arc* negative. The *Arc* positive neurons were reported as percentage of total number of neurons.

2.3.5 Statistical Analyses

All dependent variables were analyzed for normality using a Kolmogorov-Smirnov test. Fisher's LSD tests were conducted for post-hoc comparisons. Results were considered statistically significant when *p* values were less than 0.05. All data were analyzed using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corporation, Armonk, NY) or GraphPad Prism for Windows, Version 6.0 (GraphPad Software, Inc., La Jolla, CA).

In Experiment 1, an independent samples *t*-test was conducted to test the effects of consuming the 32% sucrose solution on the percentage of *Arc*-expressing neurons in dCA1. In Experiment 2, a one-way analysis of variance (ANOVA) was used to test the effect of training criterion (60 s, 30 s, vs. caged control) on sucrose-induced *Arc* induction in dCA1 neurons. An independent samples *t*-test was conducted to compare the effects of the easier and more stringent training criterion (60 s vs. 30 s) on latency to lick on the last training day and the amount of sucrose consumed on the experimental day. A Pearson correlation was computed to test the relationship between the latency to lick on the last training day and the amount of *Arc* expression on the experimental day.

Due to non-normality, a Mann-Whitney test was used to test for differences between the 30 s and 60 s training criterion groups on latency to contact the sipper tube on the experimental day. In Experiment 3, a one-way ANOVA was used to test the effect of consuming a sweetened-solution (4% sucrose, 0.2% saccharin, vs. caged control) on the number of *Arc*-expressing neurons in dCA1. Independent samples *t*-tests were conducted to assess for differences between the sucrose and the saccharin groups on the amount of each solution consumed during training, amount of test solution consumed on the experimental day, and latency to contact the sipper tube on the experimental day.

2.4 Results

2.4.1 Experiment 1

Consuming a 32% sucrose solution for 7 min induced *Arc* expression in dCA1 neurons (Figure 2.2A). Specifically, the percentage of *Arc*-expressing neurons was significantly higher in rats that had just consumed a 32% sucrose solution (Figure 2.2C) than in caged controls ($t(6) = -2.84$, $p < 0.05$; Figure 2.2B). Importantly, this sucrose-induced *Arc* expression in dCA1 neurons cannot be attributed to novelty, because the rats had consumed the sucrose solution in the same context and time for 10 days prior to the experimental day. Together these results suggest that consumption of a 32% sucrose solution is sufficient to induce synaptic plasticity in dCA1 neurons.

2.4.2 Experiment 2

The results of this experiment showed that additional training with the 32% sucrose solution decreased *Arc* expression in dCA1 neurons. That is, there was a significant main effect of training criterion on the percentage of *Arc*-expressing neurons

($F(2, 15) = 29.69$, $p < 0.0001$; Figure 2.3A). Specifically, rats trained with the easier criterion (i.e., the 60 s group) had significantly more *Arc*-expressing neurons than did rats trained with the more stringent criterion (i.e., the 30 s group; $p < 0.0005$). Consistent with the results of Experiment 1, the number of *Arc*-expressing neurons was higher in rats that had just consumed a sucrose solution than in caged controls regardless of training criterion (30 s: $p < 0.01$, 60 s: $p < 0.0001$). Importantly, these differences in the number of *Arc*-expressing neurons between the 30 s and the 60 s groups were not due to differences in latency to contact the bottle ($U(11) = 13.00$, $p > .05$; Figure 2.3B) or the amount of the sucrose solution consumed on the experimental day ($t(11) = 0.49$, $p > 0.05$; Figure 2.3C). The 30 s group did approach the sucrose bottle significantly faster than the 60 s group on the last training trial given the day before the experimental day ($t(11) = 5.54$, $p < 0.0005$; Figure 2.3D) and there was a significant positive correlation between latency to approach the bottle on the last training trial and sucrose-induced *Arc* expression ($r^2 = 0.34$, $p < 0.05$; Figure 2.3E). These findings are consistent with the hypothesis that additional exposure to the sucrose solution decreased the mnemonic demands associated with that solution, thereby producing sparse encoding of the memory of that meal in dCA1.

2.4.3 Experiment 3

The results of Experiment 3 show that orosensory stimulation is sufficient to induce *Arc* expression in dCA1 neurons ($F(2, 7) = 16.11$, $p < 0.005$; Figure 2.4A). Specifically, consuming a 4% sucrose solution or an isopreferred 0.2% saccharin solution induced nuclear *Arc* expression in dCA1 neurons ($p < 0.05$ vs. caged controls). Surprisingly, the saccharin group had a higher percentage of *Arc*-expressing neurons

than did the sucrose group ($p < 0.05$). These differences cannot be due to novelty or differences in familiarity with the solutions given that all of the rats were given 10 days of training and comparable amounts of prior exposure to both solutions. In addition, the rats preferred both solutions comparably during the training trials ($t(5) = 0.55$, $p > 0.05$; Figure 2.4B) and consumed similar amounts of the solution on the experimental day ($t(5) = 0.61$, $p > 0.05$; Figure 2.4C). Together, these findings suggest that orosensory stimulation is sufficient to induce synaptic plasticity in dCA1 neurons.

2.5 Discussion

The present study is the first to demonstrate that orosensory stimulation produced by consuming a sweetened solution induces synaptic plasticity as measured by *Arc* expression in dorsal hippocampal CA1 neurons. More specifically, the results show that compared to cage control rats, rats that consumed either sucrose (4 or 32%) or the non-caloric sweetener saccharin (0.2%) had a higher percentage of neurons in dCA1 expressing the IEG *Arc*, which is a marker of synaptic plasticity necessary for memory consolidation (Miyashita, Kubik, Haghighi, Steward, & Guzowski, 2009; Plath et al., 2006; Vazdarjanova et al., 2002). Moreover, our findings show that the amount of sucrose-induced *Arc* expression is dependent on the criterion used to train rats to consume the sucrose. That is, rats in the 30 s training criterion group, who took less time to start licking from the bottle on the last training day than rats trained with the less stringent 60 s criterion, had significantly lower sucrose-induced *Arc* expression than rats trained with the 60 s criterion. Collectively, these findings are consistent with our hypothesis that that dorsal hippocampal neurons form memory of a meal.

It is possible that a portion of the *Arc* expression that was observed was due to variables other than orosensory stimulation, such as licking and ingesting a solution. However, the difference in *Arc* expression between the 30 s and 60 s group cannot be due to such variables because the 30 s and 60 s group ingested similar amounts of sucrose on the experimental day. What differed was the criterion used to train them to consume the sucrose, suggesting that the increased *Arc* expression in the 60 s group was due to the increased mnemonic demands associated with that sucrose bout. This interpretation is consistent with previous studies showing that repeated training diminishes hippocampal involvement in a memory task (Packard & McGaugh, 1996) and with evidence showing that extensive behavioral training in the spatial water maze (Guzowski, Setlow, Wagner, & McGaugh, 2001) or a lever-pressing task (Kelly & Deadwyler, 2002, 2003), or repeated exposure to the same environment within a day (Guzowski et al., 2006) decreases behavior-induced *Arc* expression in CA1 neurons. Moreover, repeated exposure to an environment decreases the ability of that environment to increase hippocampal expression of other molecules critical for memory, such as phosphorylated cAMP response element-binding protein (pCREB; Moncada & Viola, 2006) and protein kinase M-ζ (Moncada & Viola, 2008). It will be important to determine in the future whether nutritionally balanced liquid or solid diets that typically contain protein, fat, and carbohydrates have a similar effect on dCA1 *Arc* expression.

The finding that consuming of a 0.2% saccharin solution induced *Arc* expression indicates that orosensory stimulation and possibly the hedonic value of that sweet stimulation is sufficient to induce meal-related synaptic plasticity in dCA1 neurons. This was unexpected given that consumption of 5 ml of 0.5% saccharin does not increase

Arc protein in the dorsal hippocampus of fluid-restricted rats (Morin, Quiroz, Mendoza-Viveros, Ramirez-Amaya, & Bermudez-Rattoni, 2011). Surprisingly, the present findings also showed that ingesting saccharin induced more *Arc* expression than did sucrose. This increase cannot be due to novelty or differences in the amount of prior experience because both groups had comparable exposure to sucrose and saccharin prior to the experimental day. The increase could be due to differences in licking microstructure: rats have more drinking bouts, less licks per bout, and shorter inter-bout intervals with saccharin than with sucrose even though they consume a similar total amount (J. C. Smith, Wilson, Krimm, & Merryday, 1987). Unlike sucrose, saccharin is not metabolized (Byard & Golberg, 1973; Renwick, 1985, 1986) and saccharin consumption does not induce the behavioral satiety sequence in rats (i.e., grooming/exploration and rest; Kushner & Mook, 1984). These latter differences raise the possibility that postingestive signals produced by sucrose attenuate orosensory stimulation-induced synaptic plasticity. This is unlikely, however, given that food-related signals such as the gut hormone ghrelin or the adipokine leptin enhance hippocampal functioning and morphology (Babri, Amani, Mohaddes, Mirzaei, & Mahmoudi, 2013; Carlini, Gaydou, Schioth, & de Barioglio, 2007; Carlini et al., 2004; Chen et al., 2011; Diano et al., 2006; Farr, Banks, & Morley, 2006; Garza, Guo, Zhang, & Lu, 2008; Gisou, Soheila, & Nasser, 2009; Moon, Kim, Hwang, & Park, 2009; Oomura et al., 2006). Another possibility is that extensive training with the sucrose solution attenuated sucrose-induced *Arc* in dCA1 neurons, but that the same amount of training with saccharin did not. Recent findings indicate that degree of experience with saccharin does not impact saccharin-induced

Arc protein expression in the gustatory cortex (Inberg, Elkobi, Edri, & Rosenblum, 2013).

In summary, our study demonstrates for the first time that an episode of drinking a sweetened solution induces synaptic plasticity as measured by *Arc* expression in dCA1 neurons. Combined with our previous finding showing that inactivation of dorsal hippocampal neurons induced after rats had stopped consuming a sucrose solution accelerates the onset of the next sucrose bout and increases the amount consumed (Henderson et al., 2013), our findings support the hypothesis that dorsal hippocampal neurons form a memory of a meal and inhibit meal onset during the postprandial period (Henderson et al., 2013; Parent et al., 2014). The present findings may have implications for the prevention and treatment of obesity. We and others have shown that excess intake of fats and/or sugars and obesity impair dorsal hippocampal-dependent memory (e.g., place recognition and spatial water maze; Beilharz, Maniam, & Morris, 2014; Darling, Ross, Bartness, & Parent, 2013; Erion et al., 2014; Parent et al., 2014; Pathan, Gaikwad, Viswanad, & Ramarao, 2008; Ross, Bartness, Mielke, & Parent, 2009; Ross, Bruggeman, Kasumu, Mielke, & Parent, 2012), raising the possibility that diet-induced obesity is caused and maintained by a vicious cycle wherein excess intake disrupts dorsal hippocampal function, which further increases intake (Davidson et al., 2005; Davidson, Sample, & Swithers, 2014; Kanoski & Davidson, 2011; Parent et al., 2014).

2.6 Acknowledgements

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2.7 Chapter 2 Figures

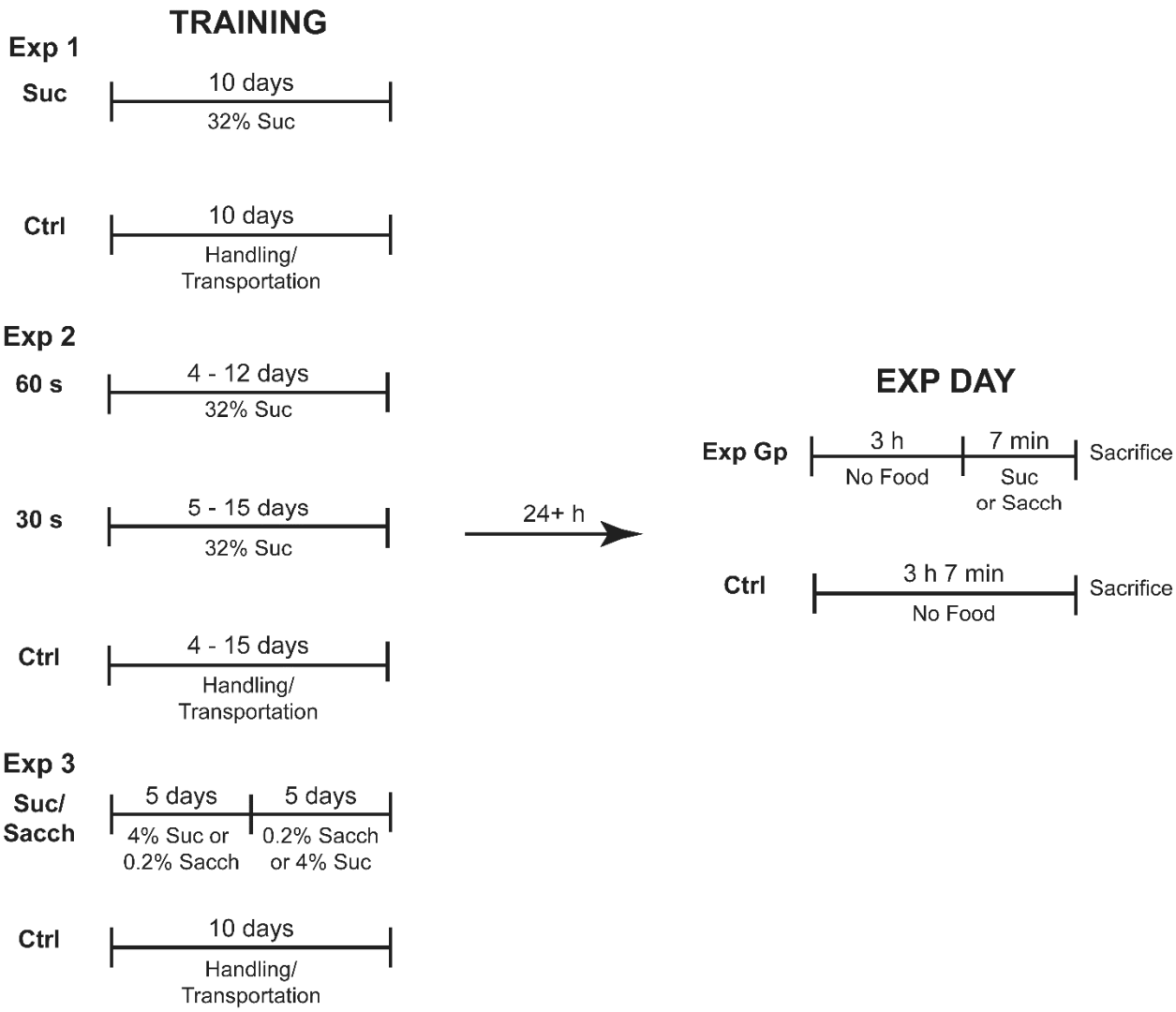

Figure 2.1 Experimental timeline

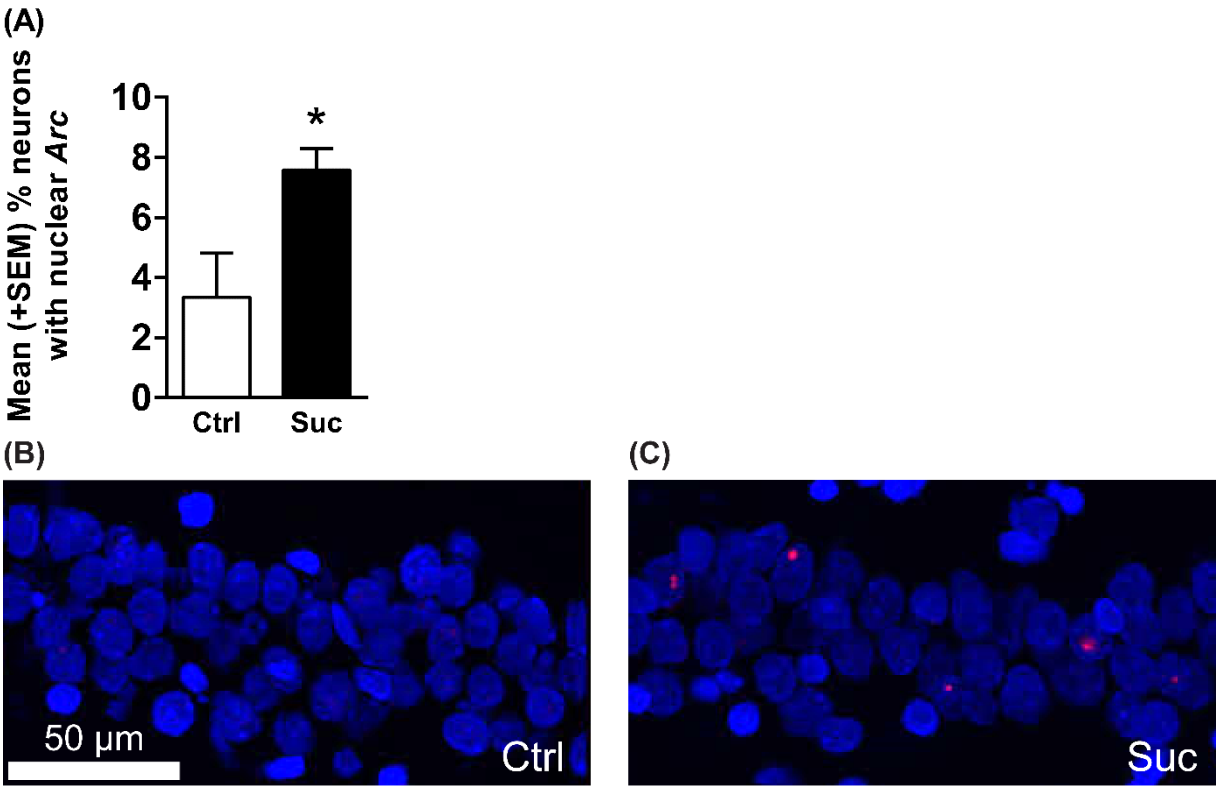

Figure 2.2 The effects of consuming a 32% sucrose solution on *Arc* expression in dCA1 neurons

(A) Consuming a 32% sucrose solution (Suc) significantly increased the percentage of neurons expressing nuclear *Arc* expression in dCA1. Sample images from the dCA1 region of a (B) caged control (Ctrl) and (C) Suc rat. Each image is a stack of six 1.5 µm-thick optical sections from the center of a 20 µm-thick stack. *Arc* mRNA is in red and nuclei stained with DAPI in blue. *$p < 0.05$ vs. Ctrl

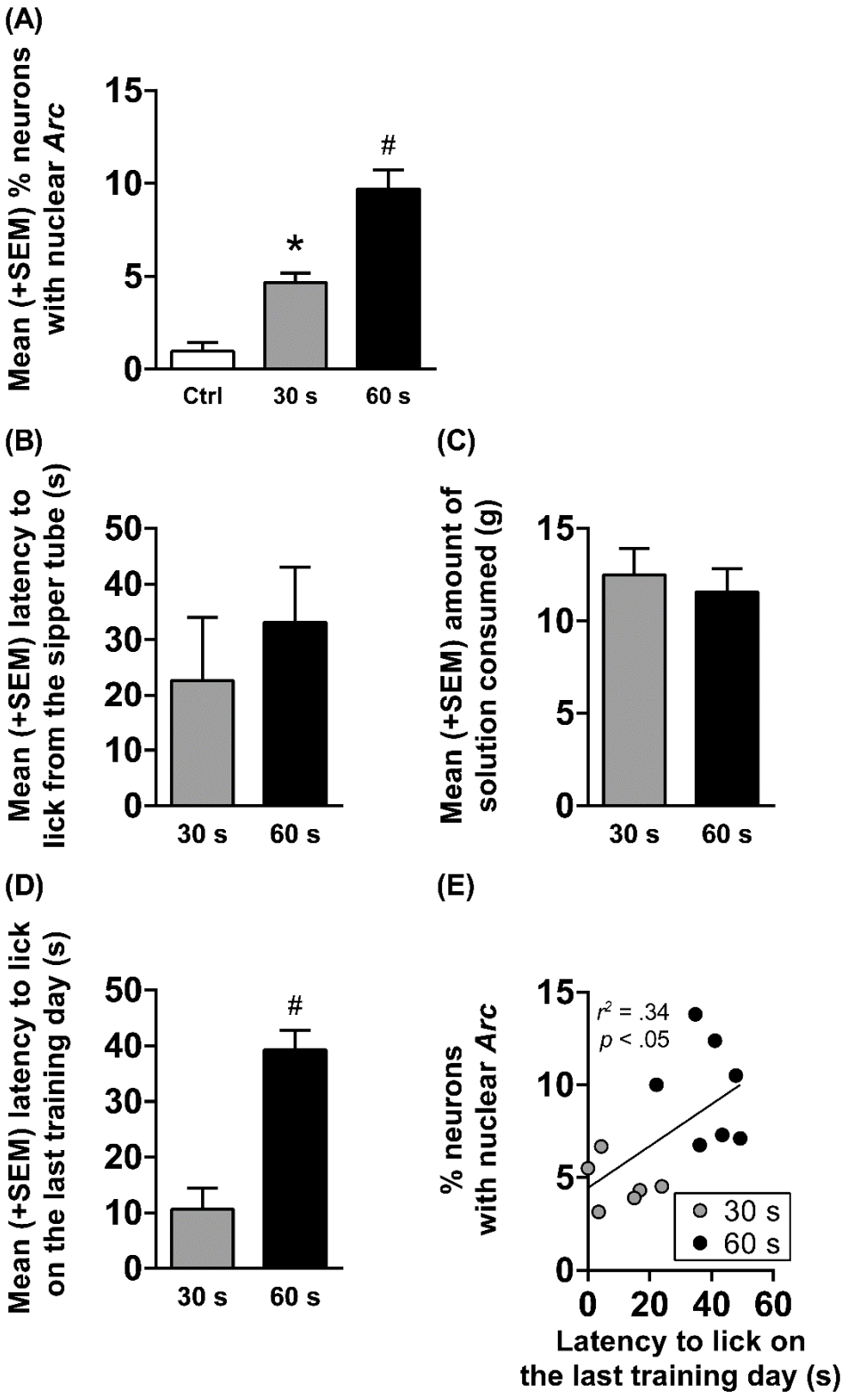

Figure 2.3 The effects of two different training protocols on sucrose-induced *Arc* expression

(A) Rats trained to consume sucrose with the less stringent 60 s training criterion (60 s) had significantly more *Arc* expression in dCA1 neurons than rats trained with the 30 s criterion (30 s). Both the 30 and 60 s group had more *Arc* expression than caged controls. The 30 and 60 s groups did not significantly differ in (B) latency to contact the bottle or (C) the amount of the sucrose solution consumed on the experimental day. (D) The 30 and 60 s groups did significantly differ in latency to contact the sipper tube on the last training trial prior to the experimental day and (E) there was a significant positive correlation between latency to contact the sipper tube on the last training trial and sucrose-induced *Arc* expression in dCA1 neurons. $^{\#}p < 0.0005$ vs. 30 s, $^{*}p < 0.01$ vs. Ctrl

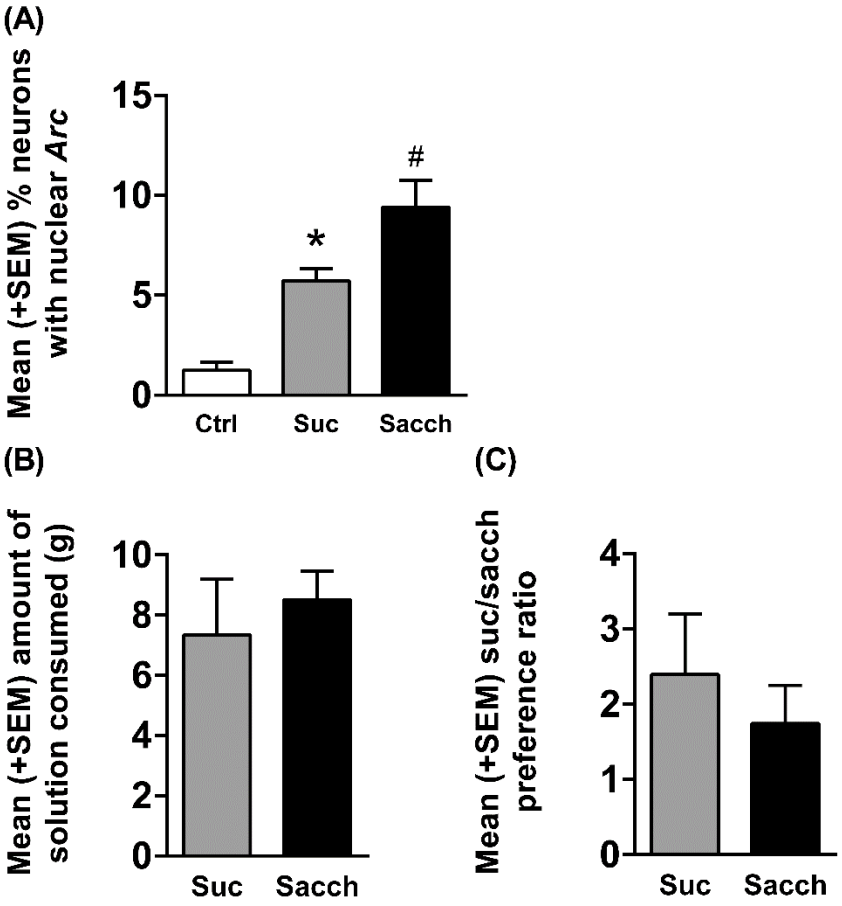

Figure 2.4 The effects of consuming a 4% sucrose or a 0.2% saccharin solution on *Arc* expression

(A) 0.2% saccharin (Sacch) produced larger increases in *Arc* expression in dCA1 neurons than 4% sucrose (Suc), which in turn produced more *Arc* expression than caged controls. The rats (B) preferred the 4% sucrose and the 0.2% saccharin solutions comparably during training and (C) consumed similar amounts of the test solution on the experimental day. $^{*}p < 0.05$ vs. Ctrl, $^{\#}p < 0.05$ vs. Suc

3 CHAPTER 3: HIPPOCAMPAL NEURONS INHIBIT MEAL ONSET

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3.1 Abstract

There is extensive research regarding the neural mechanisms involved in satiety and meal termination; in contrast, there is very limited understanding of how meal onset is regulated. On the basis of several converging lines of evidence, we hypothesized that hippocampal neurons form a memory of a meal and inhibit meal onset during the postprandial period. As a first step, we tested whether reversible inactivation of the hippocampus with muscimol infusions after the end of one meal would accelerate the onset of the next meal. To test this, adult male Sprague-Dawley rats (N = 23) were implanted with a cannula aimed at the right or left dorsal hippocampus and then trained to consume a 32% sucrose solution at a scheduled time daily. On the experimental day, hippocampal neuronal activity was temporarily disrupted during the postprandial period by infusing muscimol (0.5 μg/μl; 1 μl) 5 min after the rats stopped consuming the sucrose solution. Compared to vehicle infusions, muscimol infusions significantly decreased the latency to start the postinfusion meal and increased the size of the postinfusion meal. In addition, muscimol disrupted the relationship between the size of a

meal and length of the following postprandial period. These effects of muscimol on meal onset were not due to an effect on the speed of consumption. Collectively, these findings are consistent with the hypothesis that hippocampal neurons suppress meal initiation during the postprandial period. Given that overeating can impair hippocampal function, these findings suggest that impaired hippocampal functioning is a cause and consequence of overeating and obesity.

3.2 Introduction

A wide variety of species, including both vertebrates and invertebrates, consume food in bouts (i.e., meals; Simpson & Bernays, 1983; G. P. Smith, 2000). There is a wealth of knowledge regarding the mechanisms that determine when to stop eating (Clifton, 2000; Davis et al., 2001; G. P. Smith, 1996, 2000, 2001; Strubbe & Woods, 2004). In contrast, there is a very limited understanding of the mechanisms that control meal onset and the postprandial intermeal interval (ppIMI), which is the time spanning from the end of one meal to the beginning of the subsequent meal. What little is known is that internal hunger signals, endogenous biological clocks, and environmental and social cues associated with palatability of food are involved. For example, decreases in plasma glucose concentrations can stimulate eating (Campfield & Smith, 2003). Lesions of the suprachiasmatic nucleus, which controls circadian rhythms, disrupt meal timing (Kersten et al., 1980; Le Magnen, 1969; Van den Pol & Powley, 1979). Furthermore, the presentation of food or cues associated with palatability will cause a sated rat or human to eat, although it is not clear whether these reflect effects on the duration of the ppIMI (Birch et al., 1989; Cornell et al., 1989; Sclafani & Rendel, 1978; Weingarten, 1983).

Evidence is emerging that the hippocampus, which is a brain region important for episodic autobiographical memory (Dickerson & Eichenbaum, 2010; Düzel, Penny, & Burgess, 2010; Eichenbaum, 2004; Shapiro, Kennedy, & Ferbinteanu, 2006; Tulving, 1972; Winocur, Moscovitch, & Bontempi, 2010), is also involved in the control of eating and energy regulation (Benoit et al., 2010; Davidson et al., 2010; Davidson et al., 2007; Davidson et al., 2005; Kanoski & Davidson, 2011; Tracy et al., 2001). Hippocampal neurons receive neural signals regarding food stimuli from multiple brain regions that are important for food intake, including the arcuate nucleus of the hypothalamus, the nucleus of the solitary tract, insula, and the orbitofrontal cortex (Amaral et al., 1987; Insausti et al., 1987; Rolls, 2008; G. P. Smith, 2000; Wang et al., 2008; Wang et al., 2006). Hippocampal neurons also send extensive efferent projections to brain areas involved in energy regulation, including the hypothalamus, the nucleus accumbens, and the lateral septum (Brog et al., 1993; Cenquizca & Swanson, 2006; Groenewegen et al., 1987). Moreover, the hippocampus contains a multitude of receptors for pre- and postprandial hormones and adiposity signals, such as bombesin, ghrelin, and leptin (Lathe, 2001). Interestingly, manipulating the activity of these hormones in the hippocampus influences non–food-related memory (Harvey et al., 2006; Matsushita et al., 2003; Zhao et al., 2004). In addition, leptin infusions to the ventral hippocampus reduce the expression of a conditioned place preference for a context previously associated with food (Kanoski et al., 2011). Additional evidence in support of hippocampal involvement in food intake comes from evidence showing that enhancing memory for the most recently eaten meal decreases the amount eaten following recall of the meal (Higgs, 2002, 2008), whereas impairing encoding of a meal with distraction

increases the amount eaten after the distraction (Higgs & Woodward, 2009). Furthermore, manipulations that impair hippocampal functioning increase food intake and disrupt the ability of rats and humans to use interoceptive cues to guide behavior (Davidson et al., 2009; Davidson & Jarrard, 1993; Davidson et al., 2010; Higgs, 2008; Higgs et al., 2008; Hock & Bunsey, 1998).

There is a small body of evidence that implicates hippocampal neurons in meal onset in particular. For instance, the famous patient H.M. and other humans suffering from hippocampal-dependent memory deficits do not perceive hunger and satiety normally, do not remember eating, and will eat an additional meal when presented with food, even if they have just eaten to satiety (Hebben et al., 1985; Higgs et al., 2008; Rozin et al., 1998). Furthermore, rats with transection of the fornix or excitotoxic hippocampal lesions eat small meals more frequently than do control rats (Clifton et al., 1998; Davidson & Jarrard, 1993; Osborne & Dodek, 1986).

On the basis of the evidence reviewed above, our overarching hypothesis is that hippocampal neurons form a memory of a meal and inhibit meal onset during the postprandial period. As a first step toward testing this, we determined whether reversible inactivation of dorsal hippocampal neurons with muscimol infusions after the end of a meal would accelerate the timing of the next meal.

3.3 Materials and Methods

3.3.1 Animals

Thirty-nine male Sprague-Dawley rats (54-62 days old upon arrival; Charles River) were used in this study. They were housed individually in OptiRat cages (Animal Care Systems) on 12-h light and dark cycle (lights on 0800-2000) with free access to

pelleted chow and tap water (unless otherwise stated). The Georgia State University Institutional Animal Care and Use Committee approved all procedures.

3.3.2 Surgery

At least 5 days after their arrival, the rats were anesthetized in a plastic gas induction chamber with 5% isoflurane gas (Baxter) in 1000 ml/min of oxygen (Airgas USA, LLC). After being deeply anesthetized, they were given an injection of penicillin (1500 U im; Butler Animal Health Supply) and the anti-inflammatory analgesic flunixin meglumine (2.5 mg/kg sc; Butler Schein Animal Health). The rats were then placed on a stereotaxic instrument (David Kopf Instruments) and provided continuously with 1-3% isoflurane gas in 500 ml/min oxygen through an anesthesia mask (David Kopf Instruments). A guide cannula (3.8 mm long, 22-gauge; Plastics One) was implanted unilaterally above the left or right dorsal hippocampus in a counterbalanced order (AP: -3.8 mm, ML: ±2.8 mm, DV: -1.4 mm; Paxinos & Watson, 1998). The cannula was secured with mounting screws (Plastics One) and cranioplastic cement (DuraLay), and then a dummy cannula was inserted (Plastics One). At the end of the surgery, they were given an injection of 0.9% sterile saline (3.0 ml sc; Hospira). We elected to make unilateral infusions in order to reduce the number of animals that could be lost due to inaccurate cannula placement. Moreover, our early pilot findings suggested that unilateral infusions were sufficient to affect the ppIMI, and we have published several findings showing that unilateral infusions into dorsal hippocampus are sufficient to influence behavioral measures of memory (Degroot & Parent, 2000, 2001; Krebs-Kraft, Wheeler, & Parent, 2007; Krebs & Parent, 2005a, 2005b; Parent, Laurey, Wilkniss, & Gold, 1997).

3.3.3 Sucrose Training

After a minimum 1-week recovery period, the rats were trained to consume a 32% sucrose solution at a scheduled time (3 h after the lights were turned on). We elected to use the sucrose solution as the meal, because (1) it is palatable, (2) its stimulus qualities are specific, (3) its peripheral and central processing sites and mechanisms have been identified, (4) its concentration can be varied to manipulate postingestive consequences (Davis et al., 2000; Kirkham & Cooper, 1988; Waldbillig & Bartness, 1982), and (5) it cannot be hoarded.

On the first training day, the rats were removed from their home cages at lights-on, placed into polycarbonate experimental cages with ALPHA-dri bedding (Shepherd Specialty Papers), and brought from the vivarium to the illuminated behavioral testing suite. They were deprived of chow and tap water for 8 h and then presented with a bottle containing a 32% sucrose solution for 10 min. One hour later, they were returned to their home cages in the vivarium where chow and tap water were available ad libitum until the following day. This constituted one training trial. The rats were trained in the same manner the next day with the exception that they had continuous access to water. We removed the water on the first training day in order to increase the likelihood that rats would approach and consume the sucrose solution. On the third training day and on all following days, the rats were fasted for 3 h rather than 8 h to reduce the duration of deprivation to within the range of the normal ppIMI (Snowdon, 1969). Latency to contact the sipper tube was measured daily starting on training day 3 by YOH using a MultiTrack Stopwatch (version 2.3, freeware edition, MORIMOTO Shouji). Rats were

trained daily until their latencies were less than 30 s for 3 consecutive days (maximum 18 days).

3.3.4 Experimental Day and Intracranial Infusions

The first intracranial infusion was given at least 24 h after the training criterion was reached. The rats were placed in the experimental cages, brought to the behavioral testing suite, fasted for 3 h, and then a bottle containing a 32% sucrose solution was attached to the front of their cage. YOH observed the rats and used a timer (VWR International) to determine when a meal ended, which was operationally defined as 5 consecutive min without a sipper tube contact (Thaw, Smith, & Gibbs, 1998; Zorrilla et al., 2005). At this time, the rats were removed from the experimental cages, gently restrained, and given a unilateral injection of muscimol (0.5 µg in 1 µl; 0.25 µl/min, Sigma-Aldrich) or an equal volume of vehicle (phosphate-buffered saline, Cellgro). Evidence from other studies suggests that infusing 1 µl of muscimol into the hippocampus will inactivate an area within a 2.0 mm radius of the injection site (Arikan et al., 2002; J. H. Martin, 1991). The injection needle, which protruded 1.2 mm beyond the bottom of the guide cannula, was kept in place for 2 min after the injection was terminated to facilitate diffusion. Then the rats were returned to the experimental cages. Behavior was digitally recorded for 1 h after the infusions using a color CCTV camera (Panasonic) positioned directly above the cages. After 1 h, the rats were returned to their home cages in the vivarium, where chow and tap water were available ad libitum. YOH, who was blind to the identity of the solution, gave each rat one muscimol infusion and one vehicle infusion in a counterbalanced order at least 3 days apart.

3.3.5 Behavioral Analyses

Four trained observers blind to drug treatment manually scored the movie files using the real-time ethological recording and analysis software Hindsight for MS-DOS (version 1.5; programmed by Dr. Scott Weiss, UK). The interrater reliability coefficient ranged from 0.94 to 1.00 ($M = 0.97$) and differences were resolved by reviewing and discussing the recordings.

A sipper tube contact was operationally defined as any direct oral contact with the sipper tube longer than 3 s (Thaw et al., 1998). This criterion improved scoring reliability by virtually eliminating all sniffs as contacts. All sipper tube contacts were assumed to result in ingestion and the amount consumed was estimated indirectly by summing the duration of all sipper tube contacts during the meal (Figure 3.1A). The number of sipper tube contacts, amount consumed, and meal duration were measured for the preinfusion meal and for the postinfusion meal. The ppIMI included the time after the last sipper tube contact (5 min) and the time required for the infusion (6 min) and ended when the first sipper tube contact occurred after the infusion. The maximum amount of time allowed for each rat to initiate the postinfusion meal was 3600 s. Seven rats in the vehicle condition and one in the muscimol condition did not initiate a meal within 3600 s. A satiety ratio was calculated by dividing the duration of the ppIMI by the amount consumed in s during the preinfusion meal. This ratio gives an estimate of the postprandial satiety effect of the food consumed during the preinfusion meal (Panksepp, 1973).

3.3.6 Cannula Verification

Following completion of the experiments, the rats were anesthetized deeply with 5% isoflurane gas (Baxter) in 1000 ml/min of oxygen (Airgas USA, LLC) and euthanized by decapitation. The brains were extracted and stored in a 10% formaldehyde solution for at least 2 days, sectioned (60 μm) along the length of the cannula tract using a cryostat (Leica Microsystems), and then stained with thionin. An observer blind to the rats' behavioral results used a light microscope (Panasonic) and the Paxinos & Watson (1998) rat brain atlas to estimate the location of the cannula tip by determining the bottom of the cannula and estimating where the 1.2-mm injection needle would have terminated (Figure 3.1B). Those rats with damage to ~25% or more of dorsal hippocampus in that hemisphere (i.e., necrotic cells, blood, and/or holes) were excluded from the study, resulting in a final sample size of 23 rats.

3.3.7 Statistical Analyses

Difference scores for the muscimol and vehicle conditions were calculated for all the measures and analyzed for normality using a Kolmogorov-Smirnov test (Field, 2009). The ppIMI, the average duration of each postinfusion sipper tube contact, the amount consumed preinfusion, and the number of licks per s were analyzed using a paired samples *t*-test. Dependent variables that were not normally distributed were analyzed using a Wilcoxon signed-rank test. These included the satiety ratio, the number of postinfusion sipper tube contacts, the amount consumed in the postinfusion meal, and the postinfusion meal duration.

For the correlational analyses, the normality of the dependent variables was determined separately for the muscimol and vehicle conditions using a Kolmogorov–

Smirnov test. A two-tailed Spearman's rank correlation was used to calculate the relationship between the duration of the ppIMI and the amount consumed during the preinfusion meal. Results were considered statistically significant if *p* values were less than or equal to 0.05. All data were analyzed using PASW Statistics 18.0 (IBM).

3.4 Results

The number of days that it took rats to reach criterion during sucrose training ranged from 5 to 18 days ($M = 7.57$; SEM = 0.71). One rat did not make the criterion before the maximum number of training days (18 days).

There were no differences between the muscimol and vehicle conditions in the preinfusion meal. Specifically, paired-samples *t*-tests showed that the amount consumed during the preinfusion meal (vehicle: $M = 257.89$, SEM = 24.79, muscimol: $M = 265.09$, SEM = 18.71, $t(22) = -0.21$, $p > 0.05$) and the duration of the preinfusion meal (vehicle: $M = 415.60$, SEM = 50.70, muscimol: $M = 484.98$, SEM = 51.91, $t(22) = -1.11$, $p > 0.05$) did not differ significantly. However, inactivation of hippocampal neurons by muscimol infusions significantly decreased the ppIMI ($t(22) = 2.66$, $p < 0.05$; Figure 3.2A). Furthermore, Wilcoxon signed-rank tests showed that muscimol infusions decreased the satiety ratio ($z = -2.92$, $p < 0.01$; Figure 3.2B) and affected the postinfusion meal: They increased the amount consumed ($z = -1.95$, $p = 0.05$; Figure 3.2C), the total number of sipper tube contacts ($z = -2.35$, $p < 0.05$; Figure 3.2D), and meal duration ($z = -2.52$, $p < 0.05$; Figure 3.2E).

Postprandial inactivation of hippocampal neurons also disrupted the significant positive correlation between the amount eaten during the preinfusion meal and the ppIMI (vehicle: $r_s = 0.45$, $p < 0.05$; muscimol: $r_s = -0.15$, $p > 0.05$; Figure 3.2F).

Interestingly, paired samples *t*-tests revealed that hippocampal inactivation did not affect licking speed. That is, the average duration of each sipper tube contact during the postinfusion meal ($t(22) = -0.32$, $p > 0.05$; Figure 3.3A) and the number of licks per s ($t(14) = 0.72$, $p > 0.05$; Figure 3.3B) did not differ between the muscimol and vehicle conditions.

3.5 Discussion

These results are the first demonstration that temporary inactivation of the dorsal hippocampus by infusion of muscimol after a meal decreased the ppIMI. This is consistent with the previous reports that chronic hippocampal lesions increase the frequency of meals (Clifton et al., 1998; Davidson & Jarrard, 1993) and that fornix transection decreases the ppIMI (Osborne & Dodek, 1986). In addition, the muscimol infusions decreased the satiety ratio and abolished the positive relationship between the size of the preinfusion meal and the duration of the ppIMI (i.e., postprandial correlation; Le Magnen & Tallon, 1963, 1966). These effects of muscimol inactivation were specific because muscimol did not affect the average duration of each lick or the number of licks per second. Collectively, these results support our working hypothesis that dorsal hippocampal neurons form a memory of a meal and act to delay meal initiation during the postprandial period. This interpretation is consistent with extensive evidence indicating that hippocampal neurons encode episodic autobiographical memories (Eichenbaum, 2004; Shapiro et al., 2006) and that posttraining reversible inactivation of dorsal hippocampal neurons impairs place-avoidance memory (Cimadevilla et al., 2001; Lorenzini et al., 1996), object-place recognition memory (Oliveira et al., 2010), and spatial water maze memory (Cimadevilla et al., 2008; Holahan & Routtenberg, 2011).

The present results do not rule out the possibility, however, that hippocampal neurons delay meal onset by inhibiting memory of the satiating and rewarding postingestive consequences of a meal (Benoit et al., 2010; Davidson et al., 2009; Davidson et al., 2010; Davidson et al., 2007; Davidson et al., 2005; Kanoski & Davidson, 2011). In addition, hippocampal inactivation may have accelerated meal onset by interfering with the ability of hippocampal neurons to keep track of time (Deshmukh & Bhalla, 2003; Itskov, Curto, Pastalkova, & Buzsaki, 2011; MacDonald, Lepage, Eden, & Eichenbaum, 2011; Sinden, Rawlins, Gray, & Jarrard, 1986; Young & McNaughton, 2000). Additional experiments are needed to identify the processes that are involved in dorsal hippocampal control of meal onset.

Our results also showed that infusing muscimol into the hippocampus at the end of a meal increased the size of the next postinfusion meal. This finding was surprising, because chronic hippocampal lesions increase meal frequency but decrease meal size (Clifton et al., 1998), and fornix transection decreases the ppIMI without influencing meal size (Osborne & Dodek, 1986). It is not clear which methodological differences led to these differing effects of chronic hippocampal lesions versus acute inactivation on meal size. One critical difference is that acute inactivation essentially avoids the neural reorganization and functional compensation associated with permanent lesions. Given that centrally infused muscimol can inhibit neural activity for several hours (Arikan et al., 2002; J. H. Martin, 1991), neural activity was likely still disrupted during the consumption of the postinfusion meal. One possibility is that the muscimol-induced increase in meal size may be the consequence of increased positive feedback from the ingested food, decreased negative feedback, or both. Nonetheless, the present finding

that hippocampal inactivation decreased the ppIMI and increased meal size is consistent with the finding that chronic hippocampal lesions increase total food intake (Davidson et al., 2009; Davidson et al., 2005).

Intake varies on a meal-by-meal basis and is influenced, in part, by the size of and time since the last meal. For example, the postprandial correlation reflects the positive relationship between the amount consumed during a meal and the duration of the subsequent ppIMI (Le Magnen & Tallon, 1963, 1966). Correlations such as this are assumed to reflect the ability of animals to regulate meal intake in a flexible manner (Zorrilla et al., 2005). Permitting the rats to control the amount of sucrose consumed during the first meal revealed that hippocampal inactivation disrupts this flexibility.

The present findings show that unilateral inactivation of dorsal hippocampal neurons is sufficient to disrupt meal onset and meal size. These findings are consistent with previous results showing that unilateral dorsal hippocampal infusions influence behavioral measures of memory (Cimadevilla, Miranda, Lopez, & Arias, 2005; Cimadevilla et al., 2008; Cimadevilla et al., 2001; Degroot & Parent, 2000, 2001; Krebs-Kraft et al., 2007; Krebs & Parent, 2005a, 2005b; Parent et al., 1997). Although there are reports suggesting that there are differences in the functioning of the left and right hippocampus (e.g., (Goto et al., 2010; Klur et al., 2009), our analysis did not reveal any differences in the effects of inactivating the left versus right hemisphere on any of the measures (data not shown). It is not likely that bilateral infusions would have had a larger impact on meal onset and meal size than unilateral inactivation because unilateral and bilateral posttraining inactivation of dorsal hippocampal neurons impair

consolidation of spatial water maze memory to the same degree (Cimadevilla et al., 2008).

Along the septotemporal axis, the hippocampus can be divided into dorsal and ventral regions (M. B. Moser & Moser, 1998). Dorsal hippocampal neurons appear to be preferentially involved in cognition and memory, whereas ventral hippocampal neurons participate in emotional and affective processes (Bannerman et al., 2004; Fanselow & Dong, 2010; Kubik, Miyashita, & Guzowski, 2007; M. B. Moser & Moser, 1998). This distinction is consistent with our interpretation that inhibiting dorsal hippocampal neuronal activity decreased the ppIMI and increased the size of the next meal by disrupting the memory of the postinfusion meal. It is possible, however, that ventral hippocampal neurons are also involved in meal onset. Rats with lesions of either the dorsal or ventral hippocampus are impaired in a task that involves learning to associate internal energy states with shocks (Hock & Bunsey, 1998). Selective lesions of the ventral pole of the hippocampus increase food consumption and weight gain (Davidson et al., 2009). Infusions of leptin into the ventral hippocampus impair the expression of a conditioned place preference for a context previously associated with food and decrease the latency to run for food (Kanoski et al., 2011). Finally, ventral hippocampus neurons are the primary output area of the hippocampus to brain areas involved in eating, such as the hypothalamus and nucleus accumbens (Brog et al., 1993; Cenquizca & Swanson, 2006; Groenewegen et al., 1987). Although the effect of muscimol infusions into the ventral hippocampus on meal onset and meal size should be investigated in future studies, we elected to focus on dorsal hippocampal neurons in

the present report because of its identified role in episodic memory and to avoid motivational confounds.

In summary, the present experiments suggest that dorsal hippocampal neurons are an important component of the neural system that controls the onset and size of the meal that occurs at the end of the ppIMI. Moreover, our findings are likely relevant to clinical cases of overeating and obesity, because others and we have shown that overconsumption of sugar and/or fat impairs hippocampal-dependent memory (Darling et al., 2013; Davidson et al., 2012; Kanoski & Davidson, 2011; Ross et al., 2009; Ross et al., 2012). Collectively, these findings raise the possibility that there is a vicious cycle whereby impaired hippocampal functioning is a cause and consequence of overeating (Kanoski & Davidson, 2011).

3.6 Acknowledgements

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3.7 Chapter 3 Figures

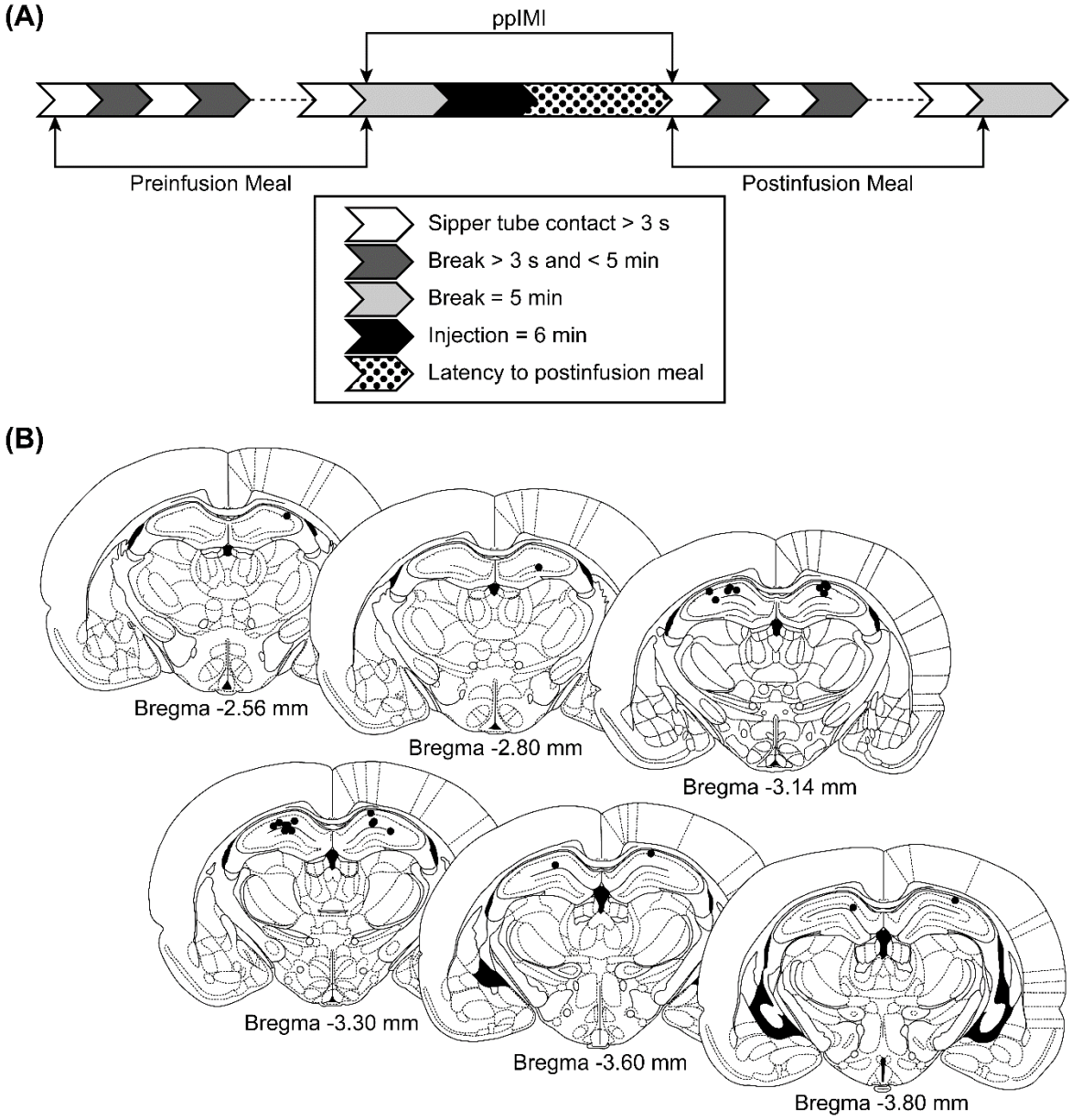

Figure 3.1 Experimental timeline and location of injection needle tips
(A) Timeline for the experimental day. (B) Estimated location of injection needle tips (Paxinos & Watson, 1998).

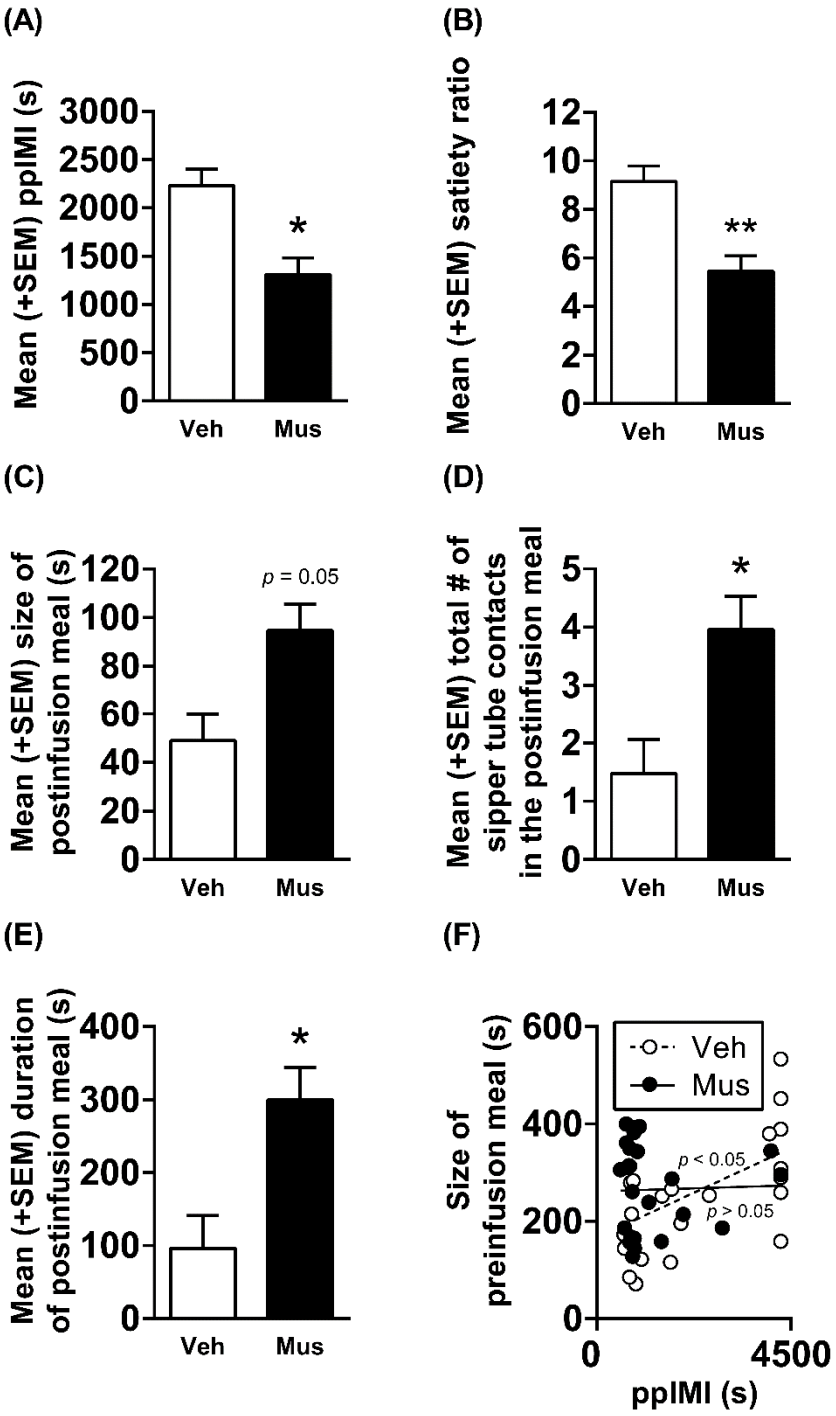

Figure 3.2 The effects of intrahippocampal muscimol infusions on meal patterning

Intrahippocampal muscimol infusions (Mus) significantly: (A) decreased the ppIMI, (B) decreased the satiety ratio, (C) increased the size of the postinfusion meal, (D) increased the total number of sipper tube contacts during the postinfusion meal, (E) increased the duration of the postinfusion meal, and (F) prevented the significant

positive correlation between the size of a meal and the following ppIMI. *$p < 0.05$, **$p < 0.01$ vs. vehicle (Veh)

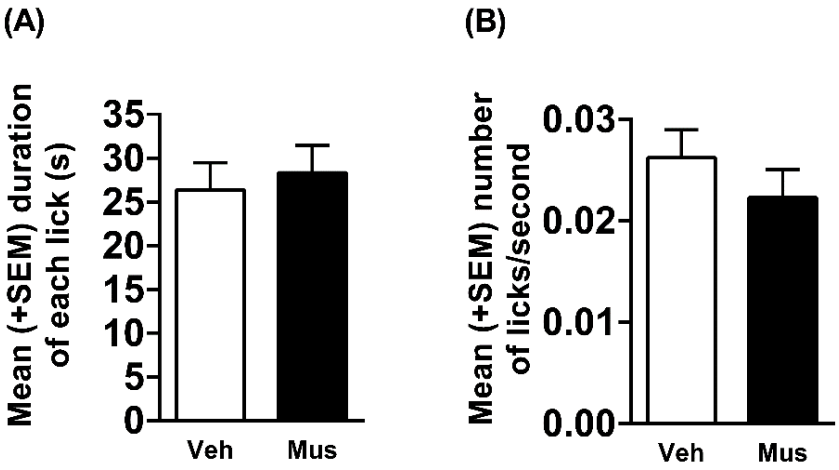

Figure 3.3 The effects of intrahippocampal muscimol infusions on licking speed
Intrahippocampal infusions of muscimol did not influence (A) the average duration of each lick and (B) the number of licks per second during the postinfusion meal.

4 CHAPTER 4: EARLY LIFE INFLAMMATORY PAIN INDUCES LONG-LASTING DEFICITS IN HIPPOCAMPAL-DEPENDENT SPATIAL MEMORY IN MALE AND FEMALE RATS

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4.1 Abstract

The present experiment tested the hypothesis that neonatal injury disrupts adult hippocampal functioning and that normal aging or chronic stress during adulthood, which are known to have a negative impact on hippocampal function, exacerbate these effects. Male and female Sprague-Dawley rats were given an intraplantar injection of the inflammatory agent carrageenan (1%) on the day of birth and their memory was tested in the hippocampal-dependent spatial water maze in adulthood and again in middle age. We found that neonatal injury impaired hippocampal-dependent memory in adulthood, that the effects of injury on memory were more pronounced in middle-aged male rats, and that chronic stress accelerated the onset of these memory deficits.

Neonatal injury also decreased glucocorticoid receptor mRNA in the dorsal CA1 area of middle-aged rats, a brain region critical for spatial memory. Morphine administration at the time of injury completely reversed injury-induced memory deficits, but neonatal morphine treatments in the absence of injury produced significant memory impairments in adulthood. Collectively, these findings are consistent with our hypothesis that neonatal injury produces long-lasting disruption in adult hippocampal functioning.

4.2 Introduction

In the United States alone, approximately 500,000 babies are born prior to 37 weeks gestation and are considered preterm (J. A. Martin et al., 2013; National Center for Health Statistics, 2014). Premature infants spend an average of 25 days in the neonatal intensive care unit (NICU), where they undergo 10-18 invasive and painful procedures each day, including endotracheal intubation, surgery, catheterization, and mechanical ventilation (Barker & Rutter, 1995; Carbajal et al., 2008; National Perinatal Information System/Quality Analytic Services, 2011; Simons et al., 2003). Although preterm infants can respond to painful stimuli (Anand & Hickey, 1987; Bartocci et al., 2006; Grunau et al., 2005; Slater et al., 2006), approximately 65% of these procedures are performed in the absence of analgesia (Bouza, 2009; Carbajal et al., 2008; Rodkey & Pillai Riddell, 2013; Simons et al., 2003; Walter-Nicolet et al., 2010).

Evidence suggests that neonatal pain activates the hypothalamic pituitary adrenal (HPA) axis. In preterm infants undergoing surgery, the opioid analgesic fentanyl significantly decreases plasma levels of stress hormones, including cortisol (Anand & Hickey, 1987), and the number of skin-breaking procedures preterm infants experience in the NICU is associated with increased cortisol levels in later development (8-18

months; Grunau et al., 2007; Grunau et al., 2004). A preclinical study on the effects of early life pain in rodents also found significantly elevated corticosterone (CORT) levels 24 h following inflammatory pain, with CORT levels remaining elevated above handled controls at 7 days post-injury (Victoria, Karom, Eichenbaum, et al., 2014).

Stress and associated high levels of CORT negatively impact the hippocampus, a brain area critical for episodic (autobiographical) memories (Eichenbaum, 2004; Shapiro et al., 2006; Tulving, 1972). Increased CORT down-regulates hippocampal glucocorticoid receptor (GR) expression (Kitraki et al., 2004), decreases dendrite number (Conrad & Bimonte-Nelson, 2010; McLaughlin et al., 2007) and synapses (Tata et al., 2006), and impairs hippocampal-dependent memory (Conrad et al., 1999; Conrad et al., 1997; McLaughlin et al., 2007; Wright & Conrad, 2005). Similarly, early life pain in rodents decreases GR mRNA and protein site-specifically in the dorsal CA1 (dCA1) region of the adult hippocampus (Victoria et al., 2013a; Victoria, Karom, Eichenbaum, et al., 2014). As increased CORT and decreased hippocampal GR expression are associated with significant memory deficits, these data suggest collectively that the stress associated with unresolved early life pain may produce long-lasting deficits in hippocampal-dependent function.

The present study assessed the impact of early life pain on adult hippocampal-dependent spatial water maze memory and GR expression in male and female rats. We further determined whether normal aging and chronic stress, life events previously shown to have a negative impact on hippocampal function, exacerbated the effects of early life pain on memory (Bizon et al., 2009; Driscoll et al., 2006; Gazova et al., 2013;

Golomb et al., 1993). The ability of morphine to attenuate the long-term consequences of early life pain on memory was also examined.

4.3 Materials and Methods

Experiment 1 tested the effects of neonatal inflammatory pain on memory in the spatial water maze in adult and middle-aged male and female rats and on hippocampal GR mRNA expression (Figure 4.1). Experiment 2 determined if chronic stress accelerated the onset of the early life pain-induced memory deficits and if analgesia given at the time of injury mitigated the impact of early life pain.

4.3.1 Animals

Pregnant Sprague-Dawley rats were received on gestational day 14 (Charles River, Wilmington, MA) and housed individually under a 12:12-h light: dark cycle with *ad libitum* access to food and water. On the day of birth (postnatal day 0; P0), pups were subjected to treatments described below. Previous studies have established that the P0 rat pup is comparable to a third trimester human infant in terms of neurodevelopment (Workman, Charvet, Clancy, Darlington, & Finlay, 2013). All experiments adhered to the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain and were approved by the Georgia State University Animal Care and Use Committee.

4.3.2 Neonatal Injury

In Experiment 1, pups were separated from their dam on P0, maintained on a warm surface, and sexed by examination of anogenital distance. Neonatal injury was induced by intraplantar administration of the inflammatory agent carageenan (CGN; 5 µL, 1% dissolved in saline; Sigma-Aldrich, St. Louis, MO). Intraplantar CGN causes paw

edema lasting approximately 48-72 h (LaPrairie & Murphy, 2007) and does not produce permanent skin, nerve or other damage in the inflamed area (Lidow, Song, & Ren, 2001; Ren, Hylden, Williams, Ruda, & Dubner, 1992; Traub, 1996). Control litters were handled in an identical manner but skin was not broken. Intraplantar saline, which has been previously shown to induce an inflammatory response (< 24 h; LaPrairie & Murphy, 2007), was not administered. Pups were separated from their dam for less than 20 min and litters were returned to their home cage as a group. Maternal behavior directed toward the injured and handled control pups is not significantly different (LaPrairie & Murphy, 2007). All pups within a litter received the same neonatal treatment and were undisturbed except for cage changes and weaning (P21).

In Experiment 2, on P0 male rat pups were given morphine sulfate (2 mg/kg, i.p.) or equivolume saline (0.9%, i.p.) 15 min prior to intraplantar CGN or handling (n = 9 handled-saline, n = 5 injured- saline, n = 5 handled-morphine, and n = 6 injured-morphine). At peak paw inflammation (5-h post-CGN), a second dose of morphine or saline was administered (LaPrairie & Murphy, 2009). Only male rats were used in Experiment 2 given our finding in Experiment 1 that there was no significant effect of injury in female middle-aged rats.

4.3.3 Estrous Cycle

Starting on P144, vaginal lavage was performed once per day in female rats for 2 weeks in the morning between 8:00 am and 10:00 am. Vaginal secretions were collected using a plastic pipette filled with deionized water. Estrus stage was defined by the presence of stage-specific epithelial cells (Becker et al., 2005) in ≥ 90% of the cell population under a light microscope (Nikon Instruments Inc., Melville, NY) as described

previously (Victoria et al., 2013a). Male rats were removed from their cage by the base of the tail, placed on a clean cart for approximately 10 s and then returned to their home cage

4.3.4 Mild Chronic Variable Stress (mCVS)

In Experiment 2, mCVS was administered to all rats starting on P94-117. On the first day of mCVS, prior to stressor exposure, blood was withdrawn (9:00 am-12:30 pm) as a measure of baseline CORT levels (Victoria, Karom, & Murphy, 2014). The mCVS protocol consisted of a daily morning (7:00 am-1:00 pm), afternoon (1:00 pm-7:00 pm), and overnight (7:00 pm-7:00 am) stress period. Different stressors were randomly administered and spanned the entire morning, afternoon, or overnight period unless otherwise specified. Stressors were: (1) water-saturated bedding, (2) restraint in an acrylic cylinder (30 min), (3) fox odor in their home cage (30 min; 1:5000; 2,4,5-trimethylthiazole; Sigma-Aldrich, St. Louis, MO), (4) cold room temperature (4 h; 4 to 6 ºC), (5) six home cage changes in 24 h, (6) insufficient home cage bedding (1:2; Bed-o'Cobs®, The Andersons, Inc., Maumee, OH), (7) white noise exposure (100 dB), (8) novel objects in their home cage (seven white golf and ping pong balls), and (9) 36 h of constant light (Morgan & Bale, 2011; Mueller & Bale, 2008). Each rat experienced all stressors two to three times. On Day 7, all rats experienced restraint stress (30 min) in the morning, forced swimming for 5 min in an inescapable water-filled pool (22 ºC) in the afternoon, and exposure to novel objects and white noise overnight to equalize stress level across the rats. On Day 8, adults were given a forced swim test (FST) and blood withdrawal (9:00 am-12:30 pm) to measure coping behavior and CORT following mCVS. The FST and CORT data are presented in a separate manuscript examining the

impact of mCVS on early life pain-induced changes in stress responses (Victoria, Karom, & Murphy, 2014).

4.3.5 Spatial Water Maze

Adult (P144-158) male (n = 14 handled; n = 14 injured) and female (n = 7 handled; n = 11 injured) rats were trained in the spatial water maze for 3 consecutive days. This task requires that rats use extra-maze cues to locate a submerged platform and is dependent on an intact dorsal hippocampus (Bannerman et al., 1999; Koh, Wheeler, & Gallagher, 2009; E. Moser, Moser, & Andersen, 1993). Rats were trained to locate a submerged clear plexiglass platform (30 cm H x 11.5 cm D; 0.5 cm below water level) in a circular water-filled (18-22 ºC) galvanized steel pool (0.61 m H x 1.4 m D) using surrounding visual cues. On training day 1, rats were placed on the platform for 30 s and then released into the pool facing the pool wall at one of three randomly selected starting locations. The experimenter gently guided the rats to the platform when the rats did not reach it within 60 s. Rats remained on the platform for 15 s, after which they were towel-dried and placed in a clear plexiglass holding cage under a heat lamp for a 30 s inter-trial interval. This constituted one training trial. During the first 2 training days, the rats were given a total of eight training trials per day, divided into two clusters of four trials separated by a 2-h break. On the third and last training day, the rats were given four training trials with no break. Latency to reach the platform during each training trial was used as a measure of acquisition. Forty-eight h after the third and final training day, a 20-s memory probe test was given. The rats were released into the pool from a novel starting location with no platform present. Swimming behavior was digitally recorded using a color CCTV camera (Panasonic System, Newark, NJ)

positioned directly above the pool. Following the memory probe test, rats were handled regularly until middle age (P424-442) but were otherwise minimally disturbed. During this interval, 5 male rats (n = 3 handled; n = 2 injured) and 2 injured female rats died. To increase statistical power, a separate cohort of injured and handled middle-aged male (n = 7 handled; n = 8 injured) and female (n = 5 handled; n = 6 injured) rats that had been trained and tested in the water maze in the same context using a different training protocol in adulthood were included, resulting in a total of 38 male (n = 18 handled; n = 20 injured) and 27 female (n = 12 handled; n = 15 injured) middle-aged rats. All rats were re-trained and re-tested in the spatial water maze task using the protocol described above. At the end of experiment, rats were decapitated and brains extracted, flash frozen in chilled 2-methylbutane (Fisher Scientific Inc., Pittsburgh, PA) and stored in -80 ºC for subsequent analysis. In Experiment 2, rats (P94-117) were trained 4-10 days following mCVS and a 30-s memory probe test was given 48 h after the last training day.

4.3.6 GR in situ Hybridization

Given that dCA1 is critical for hippocampal-dependent spatial memory (Blum, Moore, Adams, & Dash, 1999; Pittenger et al., 2002; Yiu, Rashid, & Josselyn, 2011) and that we have shown previously that neonatal injury decreases GR mRNA and protein in dCA1 (Victoria et al., 2013a; Victoria, Karom, Eichenbaum, et al., 2014), GR mRNA hybridization was measured in the present study in dCA1 (Bregma -2.52 to -4.20) of middle-aged male and female rats as described previously (Victoria et al., 2013a). Given that neonatal injury does not influence GR mRNA expression in the central amygdala (CeA; Bregma -2.40 to -3.24), this region was used as a positive

control (Victoria et al., 2013a). Fresh frozen brains were randomly selected from middle-aged handled (n = 5 male; n = 6 female) and injured (n = 5 male; n = 6 female) groups, sectioned in a 1:6 series at 20 µm, mounted on Superfrost® Plus microscope slides (Fisher Scientific Inc., Pittsburgh, PA), and stored at -80 ºC. To measure rat GR mRNA, a GR fragment was amplified from cDNA of adult prairie vole brain with rodent GR primers (forward: 5' GGACTTTCATAAAACCCTAAGGG 3'; reverse: 5' ACCCAGCAGAAAACTCCAAATCC 3'; Integrated DNA Technologies, Inc, Coralville, IA) using polymerase chain reaction. The 524 base pair nucleotide sequence of prairie vole is 90.3% identical to base pairs 97-234 and 292-680 of the rat GR sequence (*GenBank*: NM_012576). ^{35}S (PerkinElmer, Inc, Grayson, GA) UTP-labeled sense and antisense probes for GR mRNA were generated with GTP, CTP and ATP, spermidine, DTT, RNAsin and RNA polymerase, using a linearized GR template by incubating for 2 h at 37 ºC (Burkett, Spiegel, Inoue, Murphy, & Young, 2011; Inoue, Terashima, Nishikawa, & Takumi, 2004). A sense probe was used as a negative control. Sense and antisense probes were purified, dehydrated and applied to slides in hybridization buffer for 16 h at 55 ºC in a humidified chamber. Sections were washed stringently and excess probe was removed using RNAse digestion buffer containing RNAseA. Following a final high stringency wash and dehydration, sections were dried at room temperature and exposed to BioMax MR film (Sigma-Aldrich, St. Louis, MO) for 66 days for analysis and quantification.

4.3.7 Neutral Red Nuclear Staining

To determine whether changes in GR mRNA expression were due to cell loss, an adjacent series of sections was processed for the nuclear stain Neutral Red (Sigma-

Aldrich, St. Louis, MO). Sections were submerged in Neutral Red solution (1% in 0.33% sodium acetate and 0.096% glacial acetic acid buffer; 10 min) then dehydrated in a series of graded alcohols, cleared in xylene, and cover-slipped with Permount (Fisher Scientific Inc., Pittsburgh, PA).

4.3.8 Densitometry

^{14}C microscales (GE Healthcare Life Sciences, Pittsburgh, PA) with known tissue equivalent activities (disintegrations per minute per mg of tissue; dpm/mg) were used to create standard curves ($R^2 > 0.99$). Regions of interest (ROI; i.e., dCA1 and CeA) were captured with Scion Image Software (National Institute of Health, Bethesda, MD), MTI CCD 72 camera and Northern Light box (Imaging Research, Inc., Ontario, Canada). Bregma and region size from Paxinos & Watson (2005) and adjacent sections stained with Neutral Red were used for anatomical reference. For each ROI, three sections per animal were sampled randomly. The mean pixel value was recorded from a box of fixed size (dCA1: 1.0 mm^2; CeA: 1.5 mm^2). Measures were corrected for nonspecific binding by subtracting background adjacent to the ROI that lacked hybridization. Mean specific hybridization was reported as dpm/mg. For photomicrographic presentation, images were pseudocolored in Adobe Photoshop 6 (Adobe Systems Inc., San Jose, CA) using the Transparent Rainbow filter; no adjustments were made to saturation, brightness or contrast.

Neutral Red staining was also quantified in dCA1 and CeA. For each ROI, 12-bit grayscale images of three randomly selected sections were captured with a 4x objective for quantification on a Nikon Eclipse E800 microscope using a Retiga EXi CCD camera (QImaging, British Columbia, Canada) and quantified with iVision-Mac™ software

(BioVision Technologies, Exton, PA). The mean grayscale pixel value was measured from a box of fixed size as described above and recorded. Measures were corrected for nonspecific binding by subtracting background adjacent to the ROI that lacked Neutral Red uptake. Mean specific Neutral Red uptake was reported as the relative optical density.

4.3.9 Statistical analyses

The behavior analysis software TopScan (Clever Sys Inc., Reston, VA) was used to analyze the memory probe tests. All dependent variables were analyzed for normality using a Kolmogorov-Smirnov test. Non-normally distributed measures were log transformed using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corporation, Armonk, NY) or aligned rank transformed using ARTool software, Version 1.5.1 (Wobbrock, Findlater, Gergle, & Higgins, 2011). When non-normality could not be corrected, appropriate nonparametric tests were used. Results were considered statistically significant when *p* values were less than or equal to 0.05. Degrees of freedom were corrected when the assumptions of homogeneity of variance or sphericity were violated. All data were analyzed using SPSS or GraphPad Prism for Windows, Version 6.0 (GraphPad Software, Inc., La Jolla, CA).

In Experiment 1, a 2 x 2 x 2 x 20 mixed analysis of variance (ANOVA) was used to test the effects of sex, neonatal treatments, and age (between subject factors) on spatial maze acquisition across the training trials (within subject factor). In adult rats, *a priori* post hoc independent samples *t*-tests were conducted for each sex to determine the effects of neonatal injury on latency to reach the platform on the fifth trial of the first training day and the first trial on the second training day. These two time points were

separated by 2 and 24 h from the preceding trial, respectively, thereby serving as memory measures during training. To test the effects of the manipulations on the memory of the previous training given in adulthood (277-325 days previously), *a priori* post hoc independent samples *t*-tests were conducted in middle aged rats for each sex on latency to reach the platform on the first trial on the first training day (log transformed).

A 2 x 2 x 2 between subject ANOVA was used to test the effects of sex, neonatal treatments, and age on the following retention measures: (1) latency to reach the target quadrant (aligned rank transformed), (2) the number of platform location approaches (aligned rank transformed), (3) duration in the target quadrant, (4) mean distance from the platform location, and (5) mean swim speed. *A priori* post hoc independent samples *t*-tests were conducted separately for each sex and age to test the effects of neonatal injury on the retention measures listed above. Of these, latency to reach the target quadrant in adult rats was log transformed. Mann-Whitney tests were conducted separately for each sex and age to assess for differences between injured and handled rats on latency to reach the target quadrant in middle-aged rats and the number of the platform location approaches in all groups.

A 2 x 2 between subject ANOVA was used to test the effects of sex and neonatal treatments on GR mRNA expression and Neutral Red staining in middle-aged dCA1 and CeA.

In Experiment 2, a 2 x 2 x 20 mixed ANOVA was used to test the effects of neonatal injury and preemptive morphine treatments (between subject factors) on spatial maze acquisition across the training trials (within subject factor). A 2 x 2 between

subject ANOVA was used to test the effects of neonatal injury and morphine treatments on latency to reach the platform after breaks (i.e., on the fifth trial on the first training day and the first trial on the second training day) and on the following probe test retention measures: (1) latency to reach the platform location, (2) path length to reach the platform location, (3) the number of target quadrant approaches, (4) the number of platform location approaches, (5) duration in the platform location, and (6) mean swim speed. All of the retention measures were aligned rank transformed due to non-normality with the exception that the number of target quadrant approaches was normally distributed. Fisher's LSD tests were conducted for post hoc comparisons.

4.4 Experiment 1 results

4.4.1 Estrous cycling

All female rats cycled normally, with the exception of one handled and one injured rat that were acyclic for 1 week prior to spatial water maze training. These two female rats performed comparably to other rats in their treatment group (data not shown); therefore, their spatial water maze data were included in the analysis.

4.4.2 Spatial water maze: Acquisition

Neonatal injury did not affect the ability to learn platform location; however, neonatal injury did impair memory of the platform location during training in a sex-dependent manner (Figure 4.2). There was a significant main effect of training trials on latency to reach the platform ($F(13, 1317) = 18.12$, $p < 0.001$), indicating that rats learned to locate the platform across the training trials. In addition, there was a significant main effect of age on overall latency to reach the platform ($F(1, 103) = 12.20$, $p < 0.005$); middle-aged rats took less time to reach the platform than when they were

adults. Furthermore, there was a significant three-way interaction between training trials, neonatal treatments, and age on latency to reach the platform ($F(13, 1317) = 2.59$, $p < 0.005$). *A priori* post hoc comparisons revealed that adult male (P144-151) injured rats were impaired on trials following a 2-h break in training; i.e., they took longer to reach the platform than handled rats on the fifth training trial on the first training day ($t(20) = -2.78$, $p < 0.05$; Figure 4.2A) and on the first trial on the second training day following a 24 h break ($t(15) = -2.60$, $p < 0.05$; Figure 4.2A). Similarly, neonatally-injured female rats took longer to reach the platform than handled rats on the fifth training trial on the first training day ($t(16) = -2.46$, $p < 0.05$; Figure 4.2A), but did not differ on the first trial on the second training day ($t(16) = -1.37$, $p > 0.05$; Figure 4.2A). Neonatal injury also had a significant negative impact on latency to reach the platform in middle-aged rats on the first day of training, which was 277-325 days since they were last in the pool. Specifically, latency to reach the platform on the first training trial of the first training day was longer in male ($t(36) = 1.79$, $p < 0.05$) and female ($t(25) = -2.58$, $p < 0.05$; Figure 4.2B) middle-aged injured rats than in handled middle-aged rats. Together these results suggest that neonatal injury does not affect the ability of adult or middle-aged rats to navigate and learn the platform location in the spatial water maze, but does impair their ability to retain that information for long periods of time.

4.4.3 Spatial water maze: Probe memory test

Neonatal injury impaired spatial memory tested 48 h after training in a sex- and age-dependent manner. The three-way interaction between sex, neonatal treatment and age was not significant for any of the retention measures (all $p > 0.05$; Figure 4-3A.D). *A priori* post hoc comparisons revealed that middle-aged male injured rats had impaired

memory of the platform location compared to middle-aged male handled rats. Specifically, male middle-aged injured rats took longer to reach the target quadrant ($t(36) = 2.13$, $p < 0.05$; Figure 4.3A), approached the platform location fewer times ($U(36) = 113.00$, $p = 0.051$; Figure 4.3B), spent less time swimming in the target quadrant ($t(36) = 2.40$, $p < 0.05$; Figure 4.3C), and swam further away from the platform location over time ($t(36) = -2.32$, $p < 0.05$; Figure 4.3D) than handled rats. Importantly, mean swim speed during retention trial did not differ between injured and handled groups ($t(36) = -0.25$, $p > 0.05$, Figure 4.3E) suggesting that the differences on the retention measures between the injured and handled groups are not likely due to differences in motor activity. Retention deficits were only observed in the middle-aged injured male rats. There were no differences between injured and handled middle-aged female rats on any of the memory measures (all $p > 0.05$) and no deficits were observed in adult male and female injured rats (all $p > 0.05$ vs. same-sex handled group). Collectively, the results of the probe test indicate that aging exacerbates the detrimental effects of neonatal injury on spatial memory.

Floor effects may have prevented the observation of injury-induced deficits in female rats. When the effects of sex and age on all of the retention measures were analyzed for handled rats alone, we found that female handled rats took longer to reach the target quadrant ($F(1, 47) = 5.53$, $p < 0.05$; Figure 4.3A), spent less time swimming in the target quadrant ($F(1, 47) = 9.39$, $p < 0.005$; Figure 4.3C), and swam further away from the platform location over time ($F(1, 47) = 10.61$, $p < 0.005$; Figure 4.3D) than the male handled group. Thus, although the data suggest that the impairing effects of neonatal injury on memory are sex-dependent with male rats being particularly

vulnerable, the possibility remains that poor performance in female handled rats may have masked any neonatal injury-induced memory deficits.

4.4.4 GR mRNA and Neutral Red

Neonatal injury decreased GR mRNA expression in dCA1 of the middle-aged rat ($F(1, 18) = 16.47$, $p < 0.001$; Figure 4.4A). No treatment effect was observed for the CeA region ($F(1, 18) = 0.31$, $p > 0.05$; Figure 4.4A), suggesting that the effects of early life pain on GR mRNA was region-specific. Similarly, no significant differences in cell density were noted for the dCA1 ($F(1, 18) = 0.04$, $p > 0.05$; Figure 4.4B) or CeA ($F(1, 18) = 0.00$, $p > 0.05$; Figure 4.4B) suggesting that the observed decrease in dCA1 GR mRNA expression is not attributable to generalized hippocampal cell loss. Collectively, these data suggest that the neonatal inflammatory pain negatively impacts brain GR mRNA in a brain region-specific manner and that that these deficits may provide the biological bases for the impaired spatial memory retention observed in neonatally-injured animals.

4.5 Experiment 2 results

4.5.1 Spatial water maze: Acquisition

Neonatal injury (± morphine) did not affect the ability to learn the platform location during training and did not impair memory of the platform location during training. A significant main effect of training trials ($F(9, 194) = 10.87$, $p < 0.01$; Figure 4.5) was observed, but there was no effect of neonatal injury or morphine treatment on latency to reach the platform during training, including the trials given after long breaks (i.e., the fifth training trial on the first training day and the first trial on the second training day; all $p > 0.05$). These data indicate that all rats learned the platform location

comparably. mCVS disrupted spatial memory in adult handled rats. Specifically, latency to reach the platform on the fifth trial on the first training day was significantly longer in the mCVS handled saline-treated rats than in non-stressed handled rats from Experiment 1 ($t(11) = -2.29$, $p < 0.05$; Figure 4.5).

4.5.2 Spatial water maze: Probe memory test

mCVS significantly impaired spatial memory in neonatally-injured rats tested 48 h after training and morphine administration at the time of injury attenuated this effect. A significant interaction between neonatal injury and morphine treatment was observed for the following retention measures: (1) latency to reach the platform location ($F(1, 21) = 14.72$, $p < 0.005$, Figure 4.6A); (2) path length travelled to reach the platform location ($F(1, 21) = 16.86$, $p < 0.005$, Figure 4.6B); (3) the number of approaches to the target quadrant ($F(1, 21) = 13.51$, $p < 0.005$, Figure 4.6C) and (4) platform location ($F(1, 21) = 20.90$, $p < 0.005$, Figure 4.6D); and (5) duration in the platform location ($F(1, 21) = 19.25$, $p < 0.005$, Figure 4.6E). Neonatally-injured rats not given morphine took significantly longer to reach the platform location ($p < 0.05$), travelled a longer distance to reach the platform location ($p < 0.05$), made fewer approaches to the target quadrant and the platform location (both $p < 0.005$), and spent less time swimming in the platform location ($p < 0.01$) than handled-saline rats. Morphine administration at the time of injury eliminated these differences (all $p > 0.05$). Along with the minimal memory deficits observed in Experiment 1, these findings suggest that chronic stress accelerates the onset of neonatal injury-induced memory deficits in adult rats.

Interestingly, morphine administration in the absence of pain produced a significant impairment in spatial memory. Handled-morphine rats took longer to reach

the platform location ($p < 0.05$), had a tendency to travel a longer distance to reach the platform location ($p = 0.06$), made fewer approaches to the platform location ($p < 0.01$), and spent less time swimming in the platform location ($p < 0.005$) than handled-saline rats.

Swim speed was significantly slower in neonatally-injured rats ($F(1, 21) = 6.60$, $p < 0.05$, Figure 4.6F), which may have contributed to the longer latencies to the platform observed on the probe test. However, slower swim speed is unlikely to account for the deficits observed in other measures, including distance travelled to platform location, which are minimally influenced by swim speed. Moreover, the deficits produced by morphine administration alone are not due to differences in swim speed; there was no effect of morphine treatment and no significant interaction between neonatal injury and morphine treatments on swim speed for any of the measures (all $p > 0.05$). Rather, the combined pattern of findings observed with the swim speed and memory measures suggests that the impairing effects of neonatal injury and morphine reflect a memory impairment rather than a performance deficit.

4.6 Discussion

The present study is the first to show that neonatal inflammatory pain produces long-lasting alterations in hippocampal function that persist into middle age. Specifically, we demonstrate that neonatal injury impairs hippocampal-dependent spatial memory in an age-dependent manner such that memory deficits were more pronounced in middle-aged rats than adults. Neonatal injury also significantly reduced GR mRNA expression in the dCA1 region of the hippocampus of middle-aged rats. mCVS experienced during adulthood accelerated the onset of spatial memory impairment, such that the

performance deficits in the spatial memory test observed in neonatally-injured adult rats were comparable to those observed in middle-aged rats. Morphine administration at the time of injury completely reversed the memory deficits. Interestingly, administration of morphine in the absence of pain resulted in a significant impairment in spatial memory that was indistinguishable from neonatal injury alone. Collectively, these results suggest that early life pain results in a long-term disruption in adult hippocampal structure and function, and that these impairments may be reversed by morphine treatment at the time of injury.

Several lines of evidence suggest that the injury-induced decrease in dCA1 GR expression observed in the present study contributed to the hippocampal-dependent memory deficits. Increased GR expression in dCA1 and other hippocampal subfields is associated with improved memory (Escribano et al., 2009; Miyake et al., 2012; Sampedro-Piquero, Begega, & Arias, 2014); whereas, decreased hippocampal GR is associated with memory deficits (Gao, Xu, Liang, Huang, & He, 2013; Lee, Hwang, Yun, & Han, 2012). Moreover, GR knockout mice have impaired memory (Fitzsimons et al., 2013), including deficits in spatial water maze performance (Oitzl, de Kloet, Joels, Schmid, & Cole, 1997). One mechanism whereby GRs influence memory is via effects on hippocampal glutamate signaling (see review Sandi, 2011). GRs increase α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) surface trafficking and signaling in hippocampus, including in CA1 (Groc, Choquet, & Chaouloff, 2008; Karst & Joels, 2005; Krugers, Hoogenraad, & Groc, 2010; S. Martin et al., 2009). GRs are also localized in dendritic spines in CA1 (Jafari, Seese, Babayan, Gall, & Lauterborn, 2012),

and activation of hippocampal GRs enhances long-term memory by influencing synaptic plasticity (Revest et al., 2010; Roozendaal et al., 2010).

Our overarching hypothesis is that the stress associated with early life pain directly contributed to the observed deficits in spatial memory. As noted earlier, plasma CORT levels are significantly elevated in neonatally-injured rat pups for at least 1-week post-injury (Victoria, Karom, Eichenbaum, et al., 2014), and stress during the neonatal period has been shown to negatively impact adult hippocampal structure and function (Brunson, Eghbal-Ahmadi, Bender, Chen, & Baram, 2001; Cui et al., 2006; Maras & Baram, 2012; Oomen et al., 2010). For example, stress induced by poor maternal care elevates CORT release in rat pups and impairs hippocampal-dependent memory in late adulthood (Brunson et al., 2005). These deficits are paralleled by decreased hippocampal GR expression (Sutanto, Rosenfeld, de Kloet, & Levine, 1996), hippocampal dendritic atrophy, and impaired long-term potentiation (LTP; Ivy et al., 2010), a cellular mechanism critical for hippocampal-dependent memory (Bliss & Collingridge, 1993; Heynen, Abraham, & Bear, 1996).

The present finding that memory deficits were more pronounced in injured middle-aged rats suggests that normal aging exacerbates the effects of early life pain on adult hippocampal function. Normal aging decreases hippocampal volume and neurogenesis (Driscoll et al., 2006; Golomb et al., 1993) and impairs hippocampal-dependent memory (Bizon et al., 2009; Driscoll et al., 2006; Gazova et al., 2013). Moreover, previous studies have shown that neonatal stress produces spatial learning and memory deficits that only manifest later in life (Brunson et al., 2005; Ivy et al., 2010; Suri et al., 2013). For instance, poor maternal care-induced stress in rat pups increases

hippocampal corticotrophin-releasing factor, decreases dendritic arborization, and impairs LTP in middle-aged but not adult rats (Brunson et al., 2005; Ivy et al., 2010). Further, a transgenic mouse model of Alzheimer disease that impairs memory produces more prominent decreases in hippocampal GR in middle age than in adulthood (Escribano et al., 2009). As in the present findings, decreased hippocampal GR expression was not attributable to a decrease in hippocampal neuronal number (Escribano et al., 2009).

Chronic stress may have accelerated the onset of injury-induced memory deficits through a mechanism similar to that produced by normal aging. In support, chronic stress impairs hippocampal-dependent memory (Conrad, Galea, Kuroda, & McEwen, 1996; Kleen et al., 2006; Luine et al., 1994; Song, Che, Min-Wei, Murakami, & Matsumoto, 2006; Wright, Lightner, Harman, Meijer, & Conrad, 2006), attenuates hippocampal LTP (Aleisa, Alzoubi, Gerges, & Alkadhi, 2006; Pavlides, Nivon, & McEwen, 2002), and decreases hippocampal dendritic arborization (McKittrick et al., 2000), brain derived neurotrophic factor (BDNF; Aleisa et al., 2006), hippocampal GR number (Sapolsky, Krey, & McEwen, 1984), and hippocampal GR mRNA (Makino, Smith, & Gold, 1995). Chronic stress also accelerates the onset of hippocampal-dependent memory deficits and increases amyloid beta peptide and amyloid precursor protein in a transgenic mouse model of Alzheimer disease (Jeong et al., 2006). The present findings extend these previous findings by showing that chronic stress also accelerates the onset of memory deficits induced by early life pain.

Morphine administration at the time of the injury reversed the observed deficits in memory, likely due to a reduction in both the pain and stress associated with

intraplantar CGN. These results are consistent with our previous studies demonstrating that morphine administration at the time of injury reverses early life pain-induced changes in adult responses to pain- and anxiety-provoking stimuli (LaPrairie, Johns, & Murphy, 2008; Victoria, Karom, & Murphy, 2014). Although morphine administration at the time of the injury reversed the neonatal injury-induced memory deficits, the present findings demonstrate that morphine administration in the absence of pain produced memory deficits in adulthood. It is well established that systemic administration of morphine to adult rodents impairs memory in a variety of tests, including spatial water maze (Homayoun, Khavandgar, & Zarrindast, 2003; Houghoghi, Rezayof, Zyaian, & Zarrindast, 2009; Izquierdo, 1979; Saha, Datta, & Sharma, 1991; Stone, Walser, Gold, & Gold, 1991; Zhu et al., 2011). Repeated systemic administration of morphine to rat pups also disrupted inhibitory avoidance retention performance (McPherson et al., 2007) and conditioned place preference in adulthood (Boasen, McPherson, Hays, Juul, & Gleason, 2009). Here, we demonstrate for the first time that acute morphine administration to pups on the day of birth, in the absence of pain and/or stress, impairs hippocampal-dependent spatial memory in adulthood, indicating that one episode of morphine administration during the neonatal period is sufficient to produce long-lasting disruptions in hippocampal functioning. There are several mechanisms through which early life morphine may have produced deficits in adult spatial memory, including decreased hippocampal neurogenesis (Traudt et al., 2012), increased hippocampal cytokine tumor necrosis factor alpha (TNFα; Rozisky et al., 2013), and/or augmentation of the stress response. Whether acute morphine administration has similar effects is not known, although acute morphine administration to P0-27 rats increases CORT release

(Lesage, Bernet, Montel, & Dupouy, 2001; Nock, Cicero, & Wich, 2005) and sensitivity to stress (Nock et al., 2005).

The present results do not differentiate the contribution of pain and/or inflammation to the observed memory deficits. In addition to causing pain at the injection site (Fecho, Nackley, Wu, & Maixner, 2005), intraplantar CGN increases cytokine and interleukin-related gene expression, including IL-1β, in adult male rat paw tissue within 24 h of the injection (Yang, Mitchell, Keller, & Iadarola, 2007); these cytokines have the ability to cross the blood brain barrier (Banks, Farr, & Morley, 2002). Given that central administration of cytokines impairs memory, this raises the possibility that CGN-induced cytokine release contributed to the observed deficits in spatial memory (Barrientos et al., 2002; Gonzalez, Schioth, Lasaga, & Scimonelli, 2009; Goshen et al., 2007; Machado, Gonzalez, Schioth, Lasaga, & Scimonelli, 2010; Oitzl, van Oers, Schobitz, & de Kloet, 1993; Pugh et al., 1999). However, lipopolysaccharide-induced neonatal inflammation does not affect hippocampal-dependent spatial memory (Dinel et al., 2014; Harre, Galic, Mouihate, Noorbakhsh, & Pittman, 2008) or hippocampal GRs in adulthood, suggesting that inflammation alone did not contribute to the deficits observed in the present study.

In the present study, estrous cycle status was determined for all female rats. Although measures of anxiety in the elevated plus maze, inhibitory avoidance retention performance, and spatial water maze have been reported to vary across the estrous phase (Fedotova & Ordyan, 2010a, 2010b; Marcondes, Miguel, Melo, & Spadari-Bratfisch, 2001; Mora, Dussaubat, & Diaz-Veliz, 1996; Pompili, Tomaz, Arnone, Tavares, & Gasbarri, 2010), behavioral tests were not performed on specific days of the

estrous cycle due to small sample size and because training and testing took place over 5 days and thus extended across more than one estrous cycle (Westwood, 2008). Consequently, the findings do not reveal whether the effects of neonatal injury on adult spatial memory were influenced by naturally occurring changes in gonadal hormone levels.

In summary, our studies demonstrate for the first time that a single episode of inflammatory pain experienced on the day of birth significantly impairs hippocampal-dependent memory in adulthood, an effect that is exacerbated by normal aging. Additionally, these memory deficits are accompanied by a reduction in dCA1 GR expression in adult (Victoria et al., 2013a) and middle-aged rats (present findings). Importantly, our findings show that chronic stress accelerates the onset of the memory deficits and that morphine administration at the time of injury prevents these long-term memory deficits. We also made the novel discovery that acute administration of morphine to rat pups in the absence of pain negatively impacts adult hippocampal-dependent memory. Given the critical role of hippocampal function in memory, and that decreased hippocampal GR is associated with several serious mental illnesses (Raison & Miller, 2003; Webster, Knable, O'Grady, Orthmann, & Weickert, 2002), our findings suggest inflammatory pain experienced while undergoing treatment in the NICU may contribute to an increased risk of memory deficits and mental illness in this population.

4.7 Acknowledgements

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4.8 Chapter 4 Figures

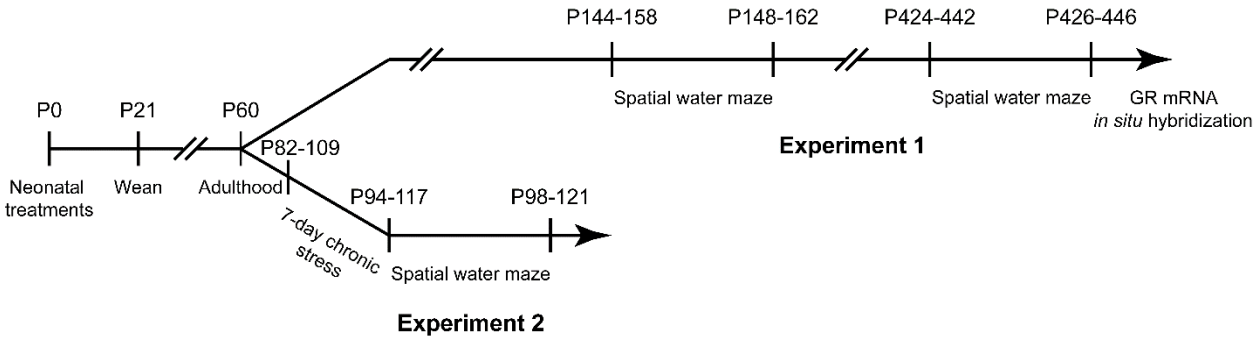

Figure 4.1 Experimental timeline

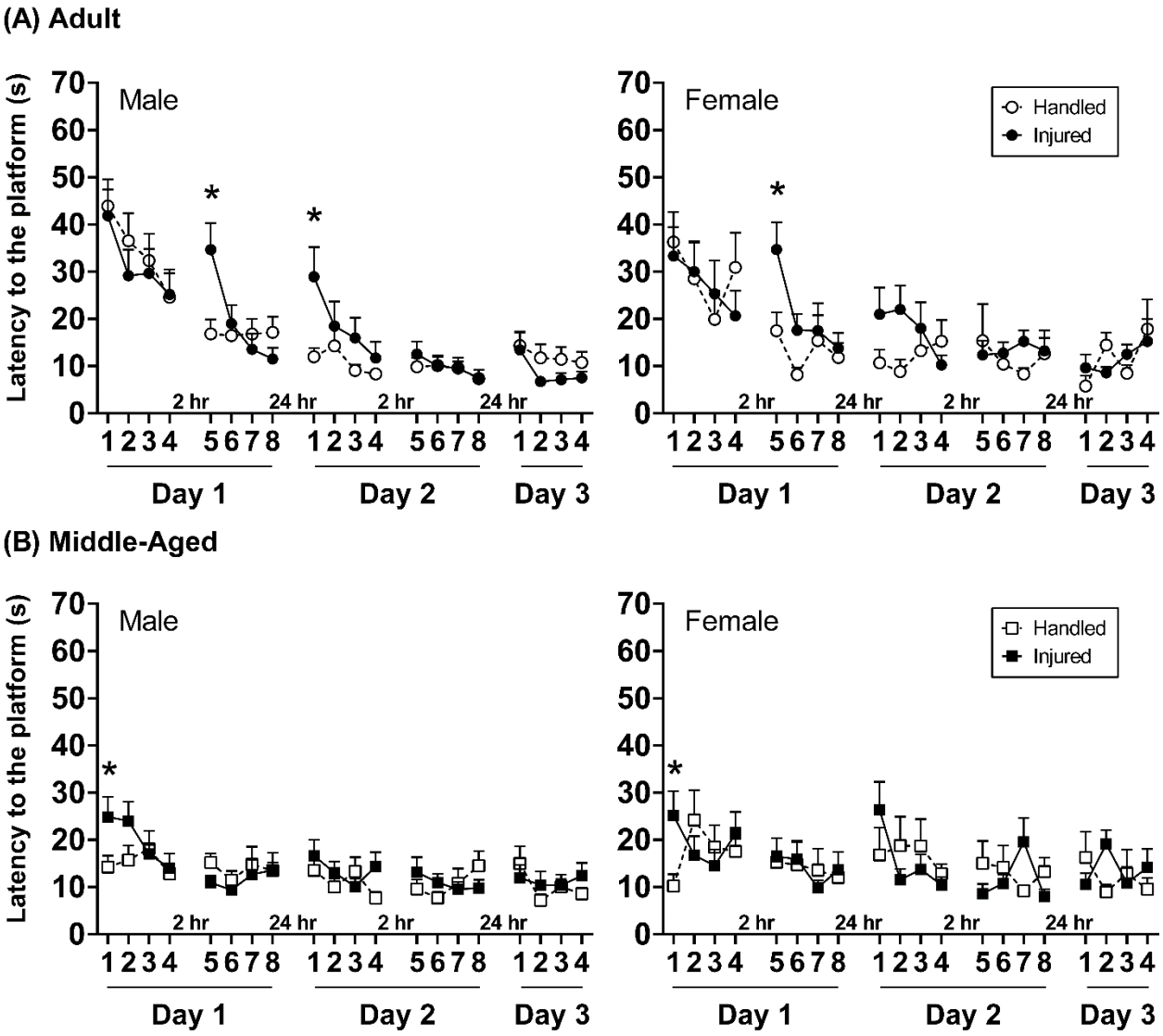

Figure 4.2 The effects of neonatal injury on latency to reach the platform during acquisition

(A) In adult rats (male P144-151; n = 14 handled, n = 14 injured; female P158; n = 7 handled, n = 11 injured) neonatal injury did not impair the ability to learn the platform location across trials, but did impair performance on trials given after a break in training. (B) Neonatal injury did not impair acquisition when rats were retrained in middle age, but impaired memory of the training given in adulthood as evidenced by longer latencies on the first training trial of the first training day in middle-aged male (P424-437; n = 18 handled, n = 20 injured) and middle-aged female rats (P439-442; n = 12 handled, n = 15 injured). *$p < 0.05$ vs. same-sex handled

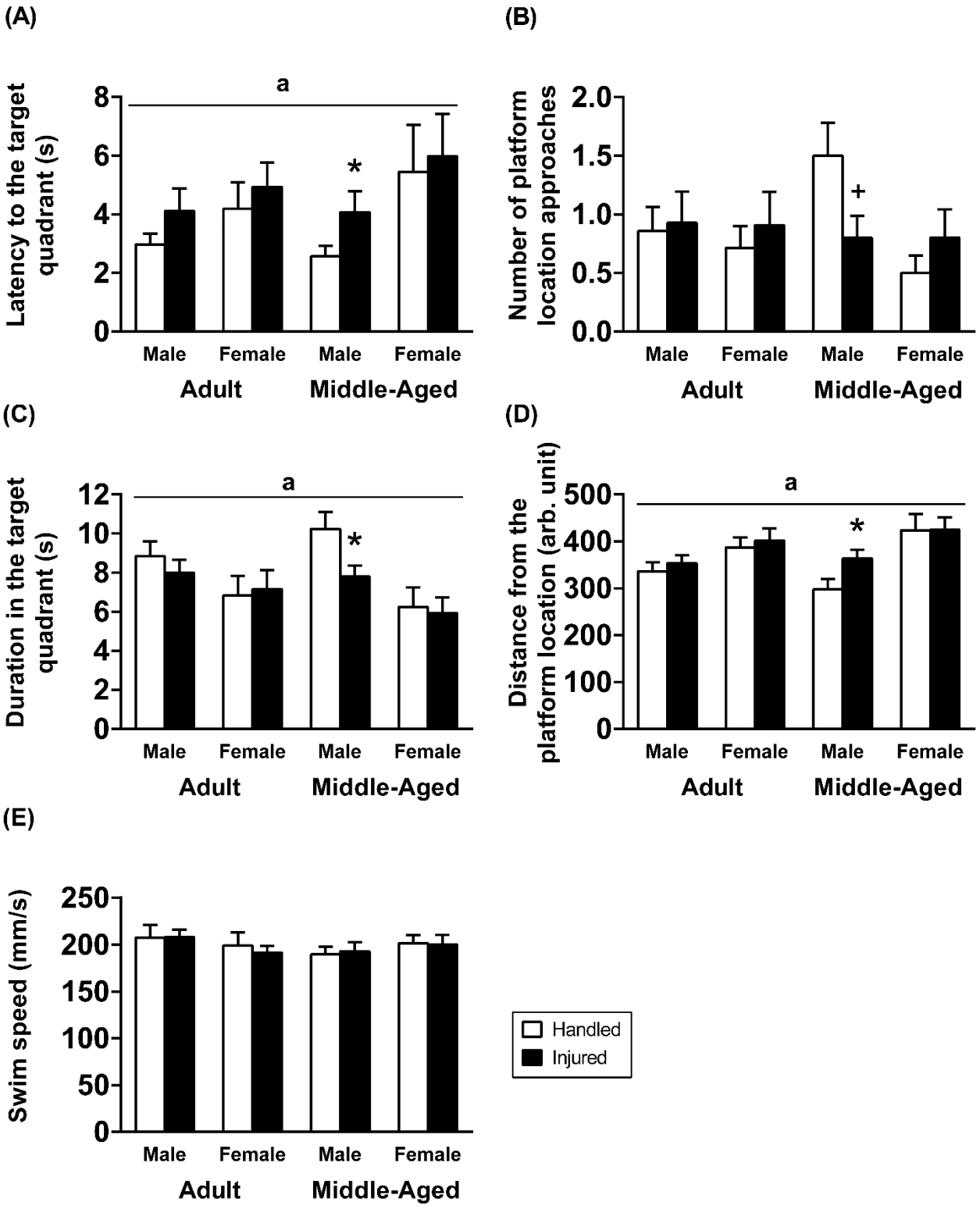

Figure 4.3 The effects of neonatal injury on long-term spatial memory in the rats when they reached middle age

Neonatal injury increased (A) latency to reach the target quadrant, decreased (B) the number of the platform location approaches and (C) time spent in the target quadrant,

increased (D) mean distance from the platform location, but did not influence (E) mean swim speed in middle-aged male rats. These differences between handled and injured rats were not observed in adult male rats, adult female rats, nor middle-aged female rats. $^{*}p < 0.05$, $^{+}p = 0.051$ vs. same-sex handled; significant main effect of sex $^{a}p < 0.05$

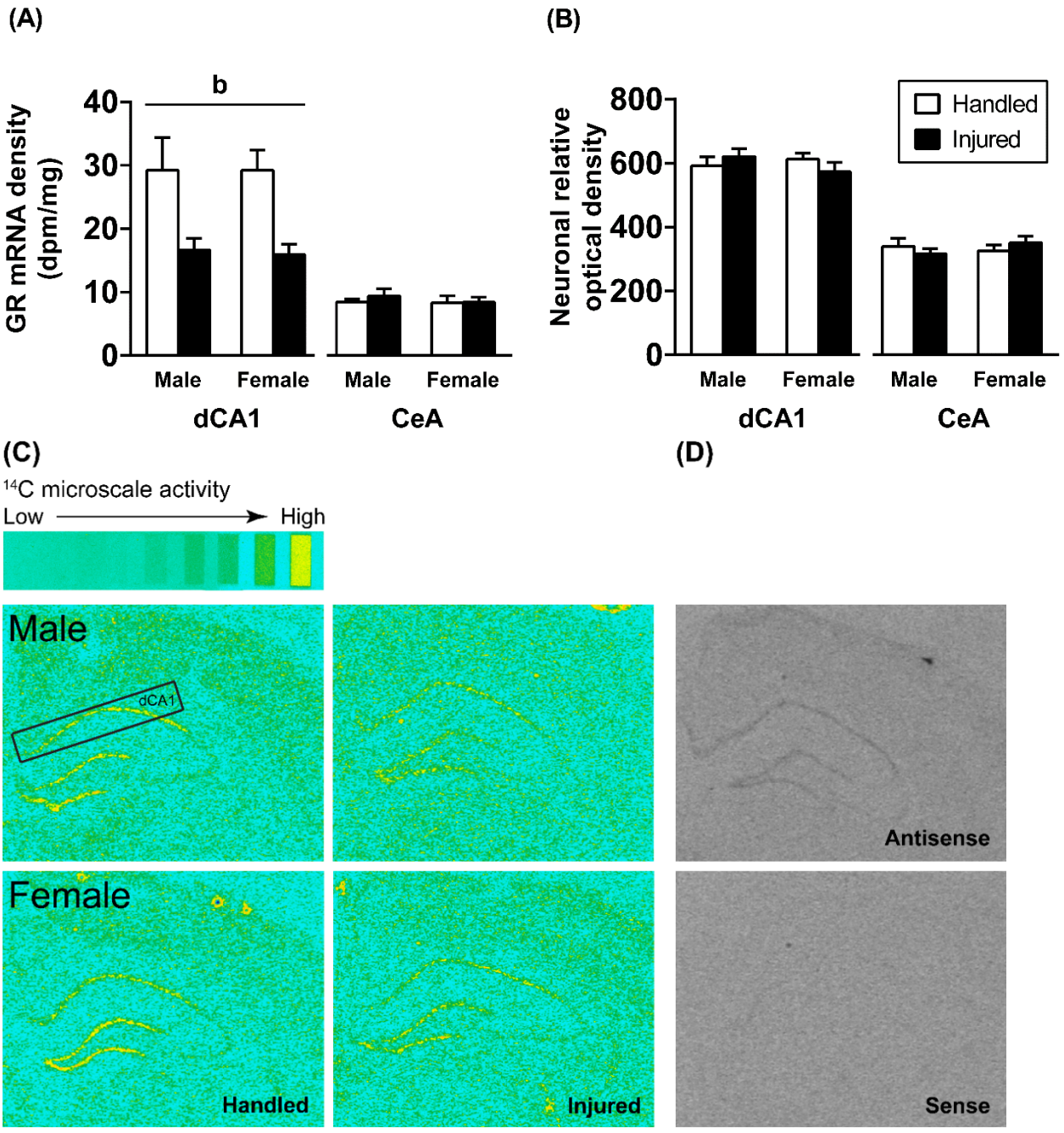

Figure 4.4 The effects of neonatal injury on GR mRNA and cell number
(A) Neonatal injury decreased GR mRNA expression in dCA1, but not in CeA in middle-aged male (P424-437; n = 5 handled, n = 5 injured) and female rats (P439-442; n = 6 handled, n = 6 injured). (B) Neonatal injury did not influence cell density in dCA1 and CeA in the same rats. Significant main effect of neonatal injury $^{b}p < 0.001$. (C) Photomicrographic presentations of GR mRNA expression in dCA1 in middle-aged male and female rats and ^{14}C microscale. (D) Oligo-antisense and sense control probes for GR mRNA.

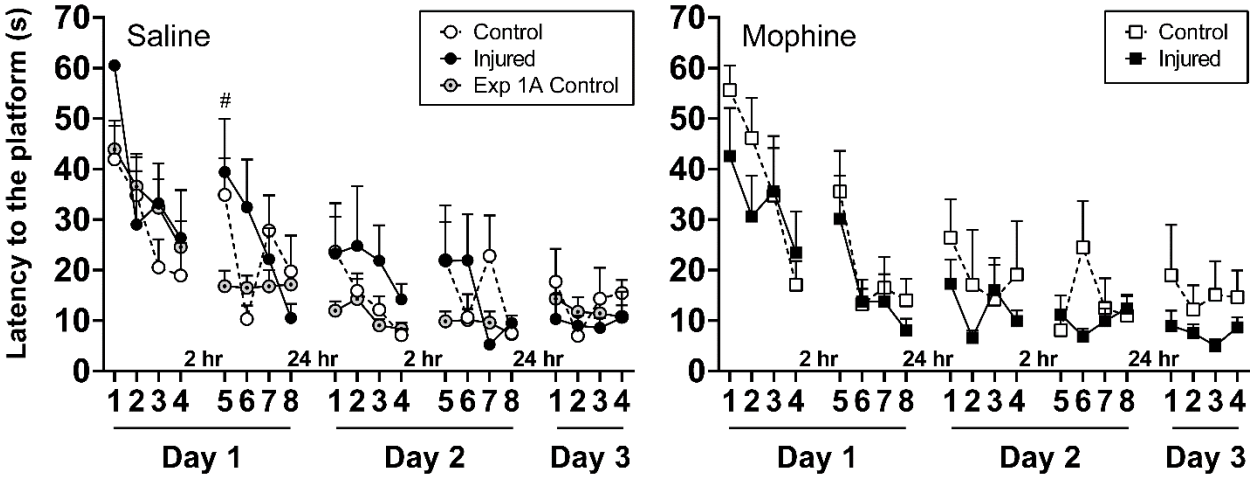

Figure 4.5 The effects of neonatal injury and morphine treatments on acquisition in adult male rats

Neonatal injury and morphine treatments did not impair acquisition and memory of the platform location during training. The hatched line depicts handled performance from Experiment 1 (P144-151; n = 14 handled). Compared to these rats, chronically-stressed handled-saline rats (P94-117; n = 9 handled-saline) in Experiment 2 had longer latencies on the fifth trial on the first training day (i.e., after the 2-h break), suggesting that the chronic stress protocol impaired memory. *$p < 0.05$ vs. Experiment 1 adult male handled

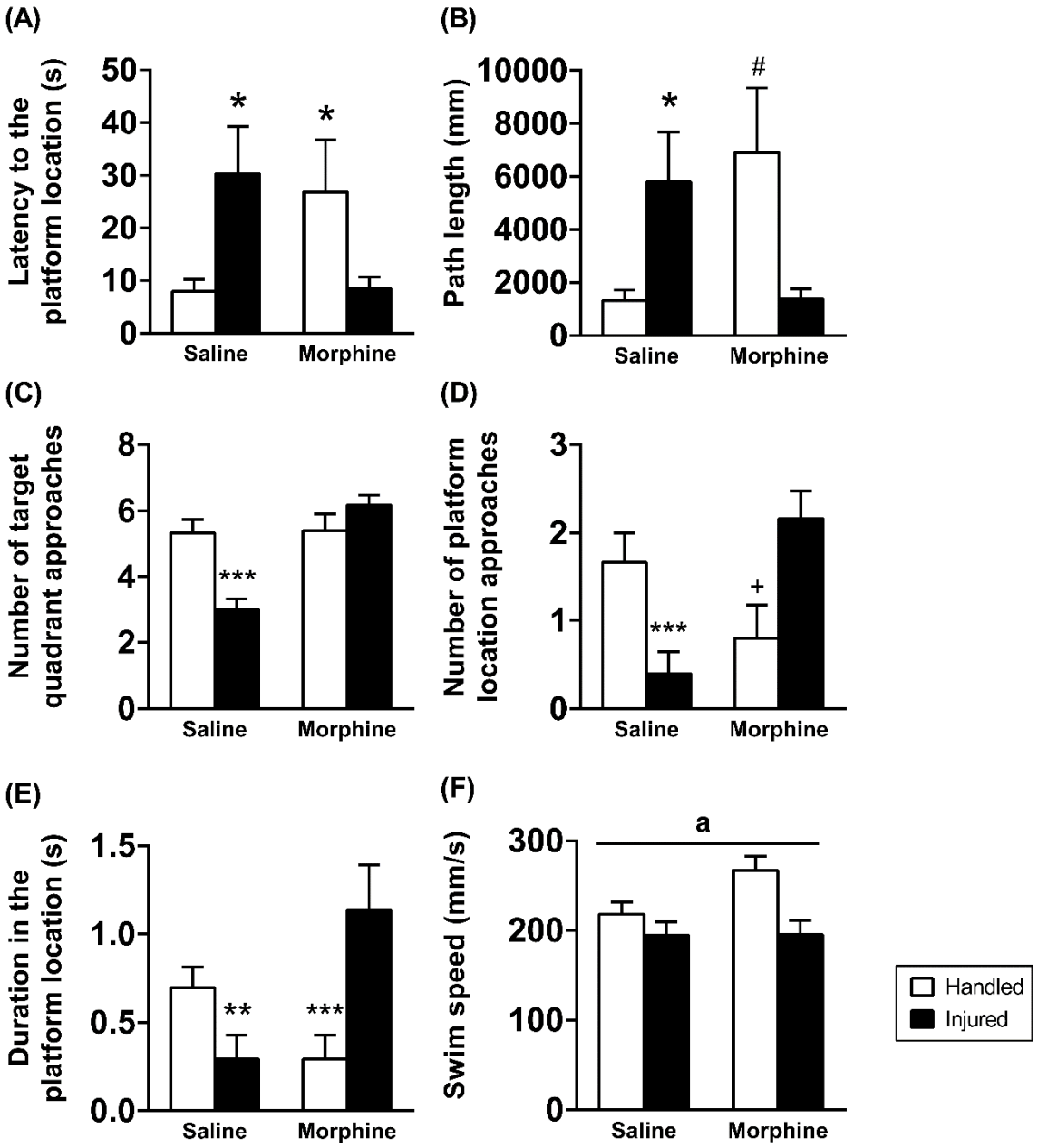

Figure 4.6 The effects of neonatal injury and morphine treatments on probe memory test in adult male rats

Neonatal injury impaired performance on the memory measures but did not affect swim speed. Specifically, injury increased (A) latency to reach the platform location and (B) path length to reach the platform location, decreased (C) the number of target quadrant approaches and (D) platform location approaches and (E) duration in the platform location, but did not affect (F) swim speed. Morphine treatments at the time of neonatal injury reversed all of the detrimental effects of injury on retention performance. Morphine treatments in the absence of pain increased (A) latency to reach the platform location, tended to increase (B) path length to reach the platform location, (D) decreased the number of platform location approaches and (E) duration in the platform

location. *$p < 0.05$, **$p < 0.01$, ***$p < 0.005$, $^{\#}p = 0.06$ vs. the handled-saline; significant main effect of neonatal injury $^{a}p < 0.05$

5 CHAPTER 5: SEX-DEPENDENT EFFECTS OF EARLY LIFE INFLAMMATORY PAIN ON ENERGY HOMEOSTASIS IN ADULT RATS

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5.1 Abstract

We hypothesize that dorsal hippocampal neurons form a memory of a meal and inhibit meal onset during the postprandial intermeal interval. In support, we have shown that eating induces expression of the synaptic plasticity marker activity-regulated cytoskeleton-associated protein (*Arc*) mRNA in dorsal hippocampal neurons and that inactivation of these neurons during the postprandial period accelerates meal onset. We recently discovered that experiencing inflammatory pain on the day of birth produces long-lasting deficits in dorsal hippocampal-dependent memory in adult rats. Based on this, the present study tested whether neonatal inflammatory pain would (1) accelerate meal onset and thereby increase meal frequency, total intake, and body mass and (2)

attenuate meal-induced synaptic plasticity in the dorsal hippocampal neurons. Male and female Sprague-Dawley rats were given an intraplantar injection of the inflammatory agent carrageenan (1%) on the day of birth and their meal patterning and body mass were measured in adulthood. We found that neonatal inflammatory pain increased meal size and meal frequency in female rats, only increased meal size in male rats, increased body mass in males and females at different ages, and attenuated sucrose-induced *Arc* expression in in female rats, but had no effect on *Arc* expression in male rats. Morphine administration at the time of injury prevented the effects of injury on sucrose intake. Collectively, these findings indicate that neonatal injury disrupts energy homeostasis in a sex-dependent manner.

5.2 Introduction

There is very limited knowledge regarding how the central nervous system controls meal onset and the interval between two meals (i.e., the postprandial intermeal interval [ppIMI]). The ppIMI is the time spanning from the end of one meal to the beginning of the subsequent meal. Evidence is emerging that the hippocampus, which is a brain region important for episodic (autobiographical) memory (Dickerson & Eichenbaum, 2010; Düzel et al., 2010; Eichenbaum, 2004; Shapiro et al., 2006; Tulving, 1972; Winocur et al., 2010), is also involved in the control of eating and energy regulation (Benoit et al., 2010; Davidson et al., 2010; Davidson et al., 2007; Davidson et al., 2005; Davidson et al., 2014; Kanoski & Davidson, 2011; Parent et al., 2014; Tracy et al., 2001). For instance, manipulations that impair hippocampal functioning increase food intake and disrupt the ability of rats and humans to use interoceptive cues to guide behavior (Davidson et al., 2009; Davidson & Jarrard, 1993; Davidson et al., 2010;

Higgs, 2008; Higgs et al., 2008; Hock & Bunsey, 1998). Furthermore, rats with transection of the fornix or excitotoxic hippocampal lesions eat more frequently than do control rats (Clifton et al., 1998; Davidson & Jarrard, 1993; Osborne & Dodek, 1986).

We hypothesize that top-down cognitive processes, such as hippocampal-dependent memory, control meal onset and thus meal frequency (Parent et al., 2014). Specifically, our overarching hypothesis is that dorsal hippocampal neurons, which are critical for episodic memory (Barbosa et al., 2012; Hoge & Kesner, 2007; Kesner et al., 2008; Li & Chao, 2008; Manns et al., 2007; Quinn et al., 2008), form a memory of a meal and temporarily inhibit meal initiation during the ppIMI. In support of this, we recently demonstrated that temporary inactivation of dorsal hippocampal neurons at the end of a sucrose meal accelerates the onset of the next sucrose meal and increases total consumption (Henderson et al., 2013). Moreover, we have also found that consuming a sucrose meal increases expression of mRNA for the immediate early gene activity-regulated cytoskeleton-associated protein (*Arc*), a neuronal marker of synaptic plasticity in dorsal hippocampal CA1 (dCA1) neurons (Chapter 2).

Findings in human participants suggest that manipulation of hippocampal-dependent memory influences meal frequency and total intake. For instance, disrupting the encoding of the memory of a meal increases the amount that is consumed at the next eating bout (Higgs & Donohoe, 2011; Higgs & Woodward, 2009; Mittal et al., 2011; Oldham-Cooper et al., 2011; Robinson et al., 2013); whereas, recalling and enhancing the memory of a recently consumed meal decreases the amount that is subsequently ingested (Higgs, 2002; Higgs & Donohoe, 2011; Robinson et al., 2013). Furthermore, the famous patient H.M. and other patients with hippocampal-dependent memory

deficits do not perceive satiety normally, do not remember eating, and will eat an additional meal when presented with food, even if they have just eaten to satiety (Hebben et al., 1985; Higgs et al., 2008; Rozin et al., 1998).

If hippocampal-dependent memory controls meal onset, then pain-induced chronic hippocampal-dependent memory impairments will increase meal frequency and total intake, thereby increasing body mass. We recently showed that an acute episode of inflammatory pain on the day of birth in rats produces long-lasting hippocampal-dependent memory deficits in adulthood (Henderson, Victoria, Inoue, Murphy, & Parent, in press). Therefore, in Experiment 1, we tested whether rats (Henderson et al., in press) had increased body mass. In Experiment 2, we tested whether neonatal inflammatory pain accelerates meal onset and diminishes meal-induced increases in *Arc* mRNA in dCA1 neurons. Furthermore, we also investigated whether morphine administration at the time of injury prevents injury-induced changes in meal patterning and in meal-induced *Arc* expression in dCA1 neurons.

5.3 Materials and Methods

5.3.1 Animals

Pregnant Sprague-Dawley rats were obtained on gestational day 14 (Charles River, Wilmington, MA) and housed individually under a 12:12-h light: dark cycle with *ad libitum* access to food and water. All experiments adhered to the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain and were approved by the Georgia State University Animal Care and Use Committee.

5.3.2 Neonatal Treatments

In Experiment 1, pups were separated from their dam on the day of birth (postnatal day 0; P0), maintained on a warm surface, and sexed by examination of anogenital distance. Neonatal injury was induced by intraplantar administration of the inflammatory agent carageenan (CGN; 5 µL, 1% dissolved in saline; Sigma-Aldrich, St. Louis, MO). Intraplantar CGN causes paw edema lasting approximately 48-72 h (LaPrairie & Murphy, 2007) and does not produce permanent skin, nerve or other damage in the inflamed area (Lidow et al., 2001; Ren et al., 1992; Traub, 1996). Previous studies have established that the P0 rat pup is comparable to a third trimester human infant in terms of neurodevelopment (Workman et al., 2013). Control litters were handled in an identical manner, but intraplantar saline was not administered because it induces an inflammatory response (< 24 h; LaPrairie & Murphy, 2007). Pups were separated from their dam for less than 20 min and litters were returned to their home cage as a group. Maternal behavior directed toward the injured and handled control pups is not significantly different (LaPrairie & Murphy, 2007). All pups within a litter were given the same neonatal treatments and were left undisturbed except for cage changes and weaning (P21). A total of 16 female (n = 7 handled, n = 9 injured) and 21 male rats (n = 10 handled, n = 11 injured) were used in Experiment 1.

In Experiment 2, in addition to the injury procedures on P0, rat pups were given morphine sulfate (2 mg/kg, i.p.) or equivolume saline (0.9%, i.p.) 15 min prior to intraplantar CGN or handling. At peak paw inflammation (5-h post-CGN), a second dose of morphine or saline was administered (LaPrairie & Murphy, 2009). A total of 67 female (n = 23 handled-saline, n = 14 handled-morphine, n = 21 injured-saline, n = 9 injured-

morphine) and 65 male rats (n = 15 handled-saline, n = 16 handled-morphine, n = 19 injured-saline, n = 15 injured-morphine) were used in Experiment 2.

5.3.3 Body Mass

In Experiment 1, body mass was measured in young adulthood (P68), in adulthood when they were tested in spatial water maze (P148-162), in middle age when they were re-tested in spatial water maze (P436), and at the time of brain collection (P456-469). See Henderson et al. (in press) for additional methodological details and spatial water maze results.

5.3.4 Sucrose Consumption

In Experiment 2, rats (male P251-255; female P242) were trained to consume a 32% sucrose solution at a scheduled time in the same location daily in order to minimize the contributions of novelty, spatial or contextual processes. We used the sucrose solution as the meal because (1) it is very palatable/rewarding to rats (Hajnal et al., 2004; G. P. Smith, 2004), (2) its stimulus qualities are more specific than meals that include fats and proteins, (3) many of its peripheral and central processing sites and mechanisms have been identified (Levine et al., 2003; G. P. Smith, 2004), (4) its concentration can be varied to manipulate postingestive consequences (Davis et al., 2000; Kirkham & Cooper, 1988; Waldbillig & Bartness, 1982), and (5) it cannot be hoarded. Importantly, we have shown previously that inactivation of hippocampal neurons after a sucrose meal accelerates the onset of the next sucrose bout (Henderson et al., 2013) and that sucrose consumption increases expression of the synaptic plasticity marker *Arc* in dCA1 hippocampal neurons (Chapter 2).

On the first training day, the rats were removed from their home cages at lights-on, placed into polycarbonate experimental cages with ALPHA-dri® bedding (Shepard Specialty Papers, Richland, MI) that did not contain food or water, and transported to the behavioral testing room. After 8 h, they were presented with a bottle containing a 32% sucrose solution for 10 min. One hour later, they were returned to their home cages where chow and tap water were available *ad libitum* until the following day. This constituted one training trial. The rats were trained in the same manner the next day with the exception that they had continuous access to water. We removed the water on the first training day in order to increase the likelihood that rats would approach and consume the sucrose solution. On the third training day and on all following days, the rats were trained in the same manner with the exception that the sucrose was given after 3 h rather than 8 h. We started with an 8-h deprivation period in order to increase the likelihood that the rats would approach the bottle, but then decreased it to 3 h in order to be within the range of an average ppIMI (Snowdon, 1969). Latency to contact the sipper tube was measured daily starting on the third training day using a MultiTrack Stopwatch, Version 2.3.1 (Morimoto Software Workshop, Japan). Rats were trained daily until their latencies were less than 30 s for 3 consecutive days (maximum 10 days).

Meal patterning was tested at least 24 h after the training criterion was reached. The rats were placed in the experimental cages, brought to the behavioral testing suite, fasted for 3 h, and then a bottle containing a 32% sucrose solution was attached to the front of their cage. An experimenter observed the rats and used a timer to determine when a meal ended, which was operationally defined as 5 consecutive min without a

sipper tube contact (Parent et al., 2014; Thaw et al., 1998; Zorrilla et al., 2005). Behavior was recorded digitally for 1 h using a color CCTV camera (Panasonic System, Newark, NJ) positioned adjacent to the cages, and then the rats were returned to their home cages in the *vivarium*, where chow and tap water were available *ad libitum*.

Two trained observers blind to the neonatal treatments manually scored the videos using the behavior observational software Stopwatch+, Version 1.5.1 (Brown, D.A., Center for Behavioral Neuroscience, Atlanta, GA). The inter-rater reliability coefficient was 1.00. A sipper tube contact was operationally defined as any direct oral contact with the sipper tube that longer than 3 s (Henderson et al., 2013; Thaw et al., 1998), which improved scoring reliability by eliminating all sniffs as contacts. All sipper tube contacts were assumed to result in ingestion and the amount consumed was estimated indirectly by summing the duration of all sipper tube contacts during the meal. Amount consumed was measured for the first and the second meal. The maximum amount of time allowed for each rat to initiate the second meal was 3600 s. To control for differences in amount eaten in the first sucrose meal, the duration of the ppIMI was estimated by calculating the satiety ratio (duration of the ppIMI divided by the amount consumed (in seconds) during the first meal (Panksepp, 1973).

5.3.5 Sucrose-Induced Arc mRNA Expression

To test the effects on neonatal injury on sucrose-induced *Arc* expression, the rats (male P315-389; female P307-401) were re-trained to consume the 32% sucrose solution as described above. At least 24 h after reaching criterion, they were allowed to consume the 32% sucrose solution for 7 min and then they were removed from the experimental cages and anesthetized in a plastic gas induction chamber with 5%

isoflurane gas (Baxter International, Deerfield, IL) in 1000mL/min of oxygen (Airgas, Inc., Radnor, PA) until they lost their righting reflex (< 1 min). They were then decapitated using a guillotine and their brains were harvested rapidly, flash frozen in chilled 2-methylbutane (Thermo Fisher Scientific, Inc., Waltham, MA), and then stored at -80 ˚C. The meal was terminated after 7 min in order to maximize the ability to detect intra-nuclear *Arc* foci that was specifically activated from consuming the solution (Vazdarjanova et al., 2002). We previously found that 7 min of sucrose consumption significantly increases *Arc* expression in dCA1 neurons (Chapter 2).

5.3.6 Fluorescence in situ Hybridization (FISH)

Right hemispheres of brains were blocked in freezing media and 20 µm coronal sections were obtained using a cryostat, mounted onto glass slides, and stored at -20 ℃ until FISH procedures were performed. The slides were processed for FISH in the following manner: After fixing the tissue in 4% paraformaldehyde and permeabilizing in a 1:1 solution of acetone and methanol, a fluorescein-labeled full-length digoxigenin-labeled *Arc* antisense riboprobe was applied and hybridized overnight at 56 ℃. Following quenching of peroxidase activity, the digoxigenin tag was revealed with peroxidase-conjugated anti-digoxigenin antibody (Roche, Indianapolis, IN) and a tyramide amplification reaction with SuperGlo™ fluorescein (Green Fluorescent Solutions, Augusta, GA). The riboprobes were generated using MAXIscript® (Ambion, Austin, TX) *in vitro* transcription kits and digoxigenin-labeled UTP (Roche, Indianapolis, IN). Nuclei were counterstained with DAPI.

5.3.7 Image Acquisition and Stereological Analysis

Image stacks from dCA1 (2.8-3.8 mm posterior to bregma) were collected from at

least three different slides from each animal using a 20× objective on a Zeiss AxioImager/Apotome system (Carl Zeiss, Dublin, CA). We focused on dCA1 because this subfield is critical for episodic memory (Barbosa et al., 2012; Farovik et al., 2010; Hunsaker et al., 2008) and because we have found that sucrose consumption increases *Arc* expression in dCA1 neurons (Chapter 2). Unbiased stereological cell counting and classification were performed as follows: (1) neuron-like cells in dCA1 in each image were segmented using an optical dissector method (West, 1999), and (2) segmented neurons were classified using Zeiss AxioVision imaging software (Carl Zeiss, Dublin, CA). Putative glial cells, which are those with small, intensely, and uniformly stained nuclei, were excluded from the analysis. Cells were classified as *Arc* positive if they contained foci of transcription for *Arc*. Cells without any foci were classified as *Arc* negative. The *Arc* positive neurons were reported as percentage of total number of neurons.

5.3.8 Statistical Analyses

All dependent variables were analyzed for normality using a Kolmogorov-Smirnov test. Non-normally distributed data were tested with appropriate nonparametric tests. Results were considered statistically significant when *p* values were less than or equal to 0.05. Degrees of freedom were corrected when the assumptions of homogeneity of variance or sphericity were violated. All data were analyzed using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corporation, Armonk, NY) or GraphPad Prism for Windows, Version 6.0 (GraphPad Software, Inc., La Jolla, CA).

In Experiment 1, a 2 x 2 x 4 mixed analysis of variance (ANOVA) was used to test the effects of sex and neonatal treatments (between subject factors) on body mass

across age (P68, 148-162, 436, and 456-469; within subject factor). Given that the memory-impairing effects of neonatal injury on hippocampal-dependent memory are sex- and age-dependent, with deficits appearing in young adulthood in female rats and in middle age in male rats (Henderson et al., in press), we predicted that (1) male neonatally-injured rats would be significantly heavier than handled rats on P436 and on P456-469 and (2) female neonatally-injured rats would be significantly heavier than handled rats on P68 and on P148-162. To test these predictions, one-tailed post-hoc independent samples *t*-tests were used to compare body mass in injured versus (vs.) same-sex handled rats. Due to non-normality, a one-tailed post-hoc Mann-Whitney test was used to compare body mass on P456-469 in male injured rats vs. handled rats.

In Experiment 2, a 2 x 2 x 2 between subject ANOVA was used to test the effects of sex, neonatal injury, and preemptive morphine treatments on the following sucrose intake measures: (1) first meal size, (2) the satiety ratio (i.e., measure of the ppIMI), (3) second meal size, and (4) total sucrose consumption on the experimental day.

We previously reported that morphine administration at the time of injury completely reversed neonatal injury-induced memory deficits, and that morphine administration in the absence of pain impaired spatial memory (Henderson et al., in press). Based on these findings, we predicted that (1) neonatal injury would disrupt meal patterning, (2) morphine administration at the time of injury would prevent these effects of injury, and (3) morphine administration in the absence of pain would produce effects comparable to those caused by injury. To test these predictions, one-tailed post-hoc independent samples *t*-tests were used to compare the intake measures in injured-saline, injured-morphine, and handled-morphine rats vs. same-sex handled-saline rats.

One-tailed post-hoc Mann-Whitney tests were used to compare the following non-normally distributed intake measures: the first meal size in female handled-morphine rats vs. handled-saline rats, the satiety ratio in female handled-morphine vs. handled-saline and in injured-morphine vs. handled-saline rats. Given that the satiety ratio and the second meal size were non-normally distributed in male handled-saline rats, all of the post-hoc comparisons on these measures were tested with Mann-Whitney tests.

A 2 x 2 x 2 between subject ANOVA was used to test the effects of sex, neonatal injury, and preemptive morphine treatments on: (1) the percentage of neurons expressing intra-nuclear *Arc* foci in dCA1, (2) latency to approach the sucrose bottle on the experimental day, and (3) the amount of sucrose solution consumed during the 7-min period. Based on our previous findings, we predicted that (1) neonatal injury would disrupt sucrose-associated *Arc* expression in hippocampal dCA1 neurons, (2) morphine administration at the time of injury would prevent these effects of injury, and (3) morphine administration in the absence of pain would parallel the effects of injury. Therefore, one-tailed post-hoc independent samples *t*-tests were used to compare the percentage of neurons expressing intra-nuclear *Arc* foci in dCA1 in injured-saline, injured-morphine, and handled-morphine rats versus same-sex handled-saline rats.

5.4 Experiment 1 Results

Neonatal injury increased body mass in a sex- and age-dependent manner. There was a significant main effect of sex on absolute body mass ($F(1, 33) = 111.76$, $p < 0.001$, Figure 5.1); overall, male rats were significantly heavier than female rats. In addition, there was a significant main effect of age on body mass ($F(2, 51) = 733.71$, $p < 0.001$), indicating that rats gained weight with age. There was also a significant

interaction between age and sex ($F(2, 51) = 84.07$, $p < 0.001$), and a significant three-way interaction between age, sex, and neonatal injury on body mass ($F(2, 51) = 3.42$, $p = 0.05$). *A priori* post-hoc comparisons revealed that male injured rats tended to be heavier than handled rats in middle age (assessed on days P436 [$t(14) = -1.56$, $p = 0.07$] and P456-469 [$U = -1.48$, $p = 0.07$]). In contrast, female injured rats were significantly heavier than handled rats as young adults (assessed on days P68 [$t(11) = -1.86$, $p < 0.05$] and P148-162 [$t(10) = -1.85$, $p < 0.05$]). Together, these results suggest that in male rats, neonatal injury induces weight gain in middle age; whereas in female rats, neonatal injury induces weight gain during young adulthood and the effect disappears with age.

5.5 Experiment 2 Results

5.5.1 Sucrose Consumption

Neonatal injury disrupted meal patterning in a sex-dependent manner. Specifically, there was a significant main effect of sex on the size of the first sucrose meal ($F(1, 123) = 35.07$, $p < 0.001$, Figure 5.2A), on the satiety ratio ($F(1, 123) = 13.76$, $p < 0.001$, Figure 5.2B), and on total sucrose consumption on the experimental day ($F(1, 123) = 30.34$, $p < 0.001$, Figure 5.2D). Compared to female rats, male rats consumed significantly more sucrose during their first meal, had a significantly decreased interval between the first and second sucrose meals as indexed by the satiety ratio (i.e., ppIMI duration/size of preceding bout), and ingested more sucrose during the experiment. In addition, there was a tendency for an interaction between neonatal injury and morphine treatment on the size of the first sucrose meal ($F(1, 123) = 3.24$, $p = 0.07$), and a significant interaction between neonatal injury and morphine

treatment on total sucrose consumption ($F(1, 123) = 3.79$, $p = 0.05$). These findings indicate that neonatal injury increased the size of the first sucrose meal as well as total sucrose consumption, but this effect of injury was not observed when morphine treatments were given at the time of injury. Specifically, *a priori* post-hoc comparisons revealed that in male rats, neonatal injury tended to increase the size of the first meal ($t(32) = -1.42$, $p = 0.08$), significantly increased the size of the second sucrose meal ($U = 95.00$, $p < 0.05$, Figure 5.2C) and increased total sucrose consumption ($t(32) = -2.35$, $p < 0.05$). Similarly, female injured rats had a significantly larger first sucrose meal ($t(42) = -2.25$, $p < 0.05$) and tended to consume more sucrose ($t(42) = -1.63$, $p = 0.06$) than female handled-saline rats. Interestingly, neonatal injury affected the satiety ratio only in female rats. Specifically, female injured rats had significantly smaller satiety ratios than handled-saline rats ($U = 167.00$, $p < 0.05$), but this effect of injury was not observed in male rats ($U = 107.00$, $p > 0.05$). These findings suggest that in male rats, neonatal injury increases meal size, whereas in female rats, neonatal injury increases meal size and also decreases the interval between two meals (i.e., the ppIMI).

Morphine administration at the time of injury eliminated the effects of injury on sucrose consumption. Specifically, the size of the first and second sucrose meal ($t(28) = 0.94$, $p > 0.05$; $U = 92.00$, $p > 0.05$, respectively) and total sucrose intake ($t(28) = 1.06$, $p > 0.05$) did not differ significantly between male injured rats given morphine at the time of injury and handled-saline rats. Similarly, the size of the first meal ($t(10) = -0.03$, $p > 0.05$), total sucrose consumption ($t(30) = 0.55$, $p > 0.05$) and the satiety ratio ($U = 89.00$, $p > 0.05$) did not differ significantly between female injured-morphine rats and handled-saline rats.

Morphine administration in the absence of pain on the day of birth impacted the satiety ratio in adult male rats. Specifically, handled-morphine rats had a significantly higher satiety ratio than handled-saline rats ($U = 77.00$, $p < 0.05$). However, morphine administration did not affect the satiety ratio in female rats or any other sucrose consumption measures in male and female rats (all $p > 0.05$).

5.5.2 Sucrose-Induced Arc mRNA Expression

Neonatal injury attenuated sucrose-induced *Arc* expression in dCA1 neurons and morphine treatments at the time of injury prevented the injury-induced decrease in a sex-dependent manner. There were significant main effects of sex and neonatal injury on sucrose-induced *Arc* expression ($F(1, 113) = 5.75$, $p < 0.05$; $F(1, 113) = 5.62$, $p < 0.05$; respectively, Figure 5.3A), indicating that sucrose induced significantly more *Arc* expression in dCA1 neurons of female rats than in male rats. Neonatal injury significantly attenuated sucrose-induced *Arc* expression in female rats ($t(38) = 1.75$, $p < 0.05$), but not in male rats ($t(30) = 0.78$, $p > 0.05$). Importantly, morphine treatments at the time of injury prevented this effect of injury in female rats: sucrose-induced *Arc* expression in dCA1 neurons did not significantly differ between female injured-morphine and handled-saline rats ($t(29) = 0.93$, $p > 0.05$).

There was a significant main effect of sex on the amount of sucrose consumed during the 7-min period before the brain collection ($F(1, 113) = 38.11$, $p < 0.001$, Figure 5.3B), revealing that male rats consumed significantly more sucrose solution than female rats during that 7-min period. In addition, there was tendency for a main effect of neonatal injury on total sucrose intake ($F(1, 113) = 3.18$, $p = 0.08$), indicating that injured rats tended to consume more sucrose than non-injured rats.

5.6 Discussion

The present study is the first to show that neonatal inflammatory pain impairs energy homeostasis in adulthood in a sex-dependent manner. Neonatal injury increased body mass in both male and female rats, but the timing of these increases differed between males and females. In female rats, neonatal injury increased body mass in adulthood but not in middle age; whereas in male rats, neonatal injury increased body mass in middle age, but not at earlier ages. Neonatal injury increased total sucrose intake in male and female rats, but these increases were due to increased meal size and accelerated meal onset in female rats (i.e. decreased ppIMI); whereas, injury only affected meal size in male rats. Neonatal injury also affected sucrose-associated synaptic plasticity in hippocampal dCA1 neurons in a sex-dependent manner. Specifically, injury attenuated sucrose-induced *Arc* expression in female rats, but had no effect in male rats. The results also show that morphine administration at the time of injury prevented injury-induced effects on sucrose intake and sucrose-associated *Arc* expression, suggesting that experiencing inflammatory pain and stress produced these deficits. Collectively, these results suggest that early life inflammatory pain promotes energy intake and increases body mass in adulthood in a sex-dependent manner and that these changes are prevented by morphine treatment at the time of injury.

The results of the present study parallel the hippocampal-dependent memory deficits that were observed in our previous study (Henderson et al., in press). Specifically, female injured rats in Experiment 1 of the present study were impaired in the spatial water maze task as young adults, but not in middle age (Henderson et al., in press). Notably, the present findings show that the ages at which they had memory

deficits (P144-162; Henderson et al., in press) are the same as when they weighed more (P68-162; present study). Similarly, hippocampal-dependent memory deficits in male rats were most pronounced when they were in middle age (P424-446; Henderson et al., in press), which is also when they weighed more (P436-469). The finding that morphine administration at the time of injury prevented the effects of neonatal injury on adult energy intake also parallels our previous finding that preemptive morphine treatments prevented injury-induced hippocampal-dependent memory deficits in adulthood (Henderson et al., in press). Furthermore, our finding that adult female injured rats in Experiment 2 had decreased satiety ratio and increased sucrose intake also parallels hippocampal-dependent memory deficits that were observed in female injured rats in young adulthood (Henderson et al., in press). The finding that neonatal injury did not affect meal onset in male rats could be due to the fact that their sucrose intake was tested well before the age at which males were previously shown to develop hippocampal-dependent memory deficits (Henderson et al., in press). That is, it is possible that the effects of injury on the satiety ratio would be apparent in male injured rats if they were tested in middle age.

Several lines of evidence suggest that the injury-induced decrease in dCA1 GR expression contributed to the hippocampal-dependent memory deficits in neonatally-injured rats (Henderson et al., in press). In our previous study, plasma corticosterone (CORT) levels were significantly elevated in neonatally-injured rat pups for at least 1-week post-injury (Victoria, Karom, Eichenbaum, et al., 2014), and stress during the neonatal period has been shown to negatively impact adult hippocampal structure and function (Brunson et al., 2001; Cui et al., 2006; Maras & Baram, 2012; Oomen et al.,

2010). For example, stress induced by poor maternal care elevates CORT release in rat pups and impairs hippocampal-dependent memory in middle-aged rats (Brunson et al., 2005). These deficits are paralleled by decreased hippocampal GR expression (Sutanto et al., 1996), hippocampal dendritic atrophy, and impaired long-term potentiation (LTP; Ivy et al., 2010), a cellular mechanism critical for hippocampal-dependent memory (Bliss & Collingridge, 1993; Heynen et al., 1996). Increased GR expression in dCA1 and other hippocampal subfields is associated with improved memory (Escribano et al., 2009; Miyake et al., 2012; Sampedro-Piquero et al., 2014); whereas, decreased hippocampal GR is associated with memory deficits (Gao et al., 2013; Lee et al., 2012). Moreover, GR knockout mice have impaired memory (Fitzsimons et al., 2013), including deficits in spatial water maze performance (Oitzl et al., 1997).

The effects of neonatal injury on hippocampal-functioning and meal patterning may also involve an effect of injury on other brain regions in addition to the hippocampus. We reported previously that neonatal injury produced cellular changes in several brain areas, including the paraventricular nucleus of the hypothalamus, the periaqueductal gray, the lateral septum, the amygdala (basolateral, cortical, and central; LaPrairie & Murphy, 2009; Victoria et al., 2013a; Victoria, Inoue, Young, & Murphy, 2013b), and induced changes in adult responses to pain- and anxiety-provoking stimuli (LaPrairie et al., 2008; Victoria et al., 2013a; Victoria, Karom, & Murphy, 2014). Importantly, manipulations of many of these brain areas (e.g., the lateral septum, the basolateral and central amygdala, and the paraventricular nucleus of the hypothalamus) influence eating and drinking behavior (Bakshi, Newman, Smith-Roe, Jochman, & Kalin, 2007; Galaverna et al., 1993; Leibowitz, Hammer, & Chang, 1981; Parker & Coscina,

2001; Rolls & Rolls, 1973). Furthermore, the finding that morphine administration at the time of injury prevented all of these changes (LaPrairie et al., 2008; Victoria, Karom, & Murphy, 2014) raises the possibility that the effects of neonatal injury on energy homeostasis also involves disrupted functioning in these brain areas.

The present findings raise the possibility that early life stress or trauma (e.g., neonatal injury) contributes to the development of obesity. Consistent with this, clinical studies show that preterm infants are likely to gain weight and/or develop obesity-related complications such as metabolic syndrome later in life (Dulloo, Jacquet, Seydoux, & Montani, 2006; Euser et al., 2005; Fagerberg, Bondjers, & Nilsson, 2004; Finken et al., 2006; Hofman et al., 2004; Singhal, Fewtrell, Cole, & Lucas, 2003; Uthaya et al., 2005; Yeung, 2006). Furthermore, experiencing physical and/or psychological stressors during early stage of life increases adipose tissue mass and induces hyperinsulinemia in rats (Haley et al., 2013) and is associated with obesity and metabolic syndrome in adulthood in humans (D'Argenio et al., 2009; Gunstad et al., 2006; Rohde et al., 2008; Vanitallie, 2002).

In summary, our studies demonstrated for the first time that a single episode of inflammatory pain experienced on the day of birth disrupts energy homeostasis and meal-related hippocampal synaptic plasticity in a sex and age-dependent manner. Importantly, our findings show that morphine administration at the time of injury prevents these effects. Together, our findings suggest that inflammatory pain experienced while undergoing treatment in the NICU may contribute to an increased risk of obesity in this population and that preventing pain in these infants in NICU may reduce their increased risk for obesity.

5.7 Acknowledgements

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5.8 Chapter 5 Figures

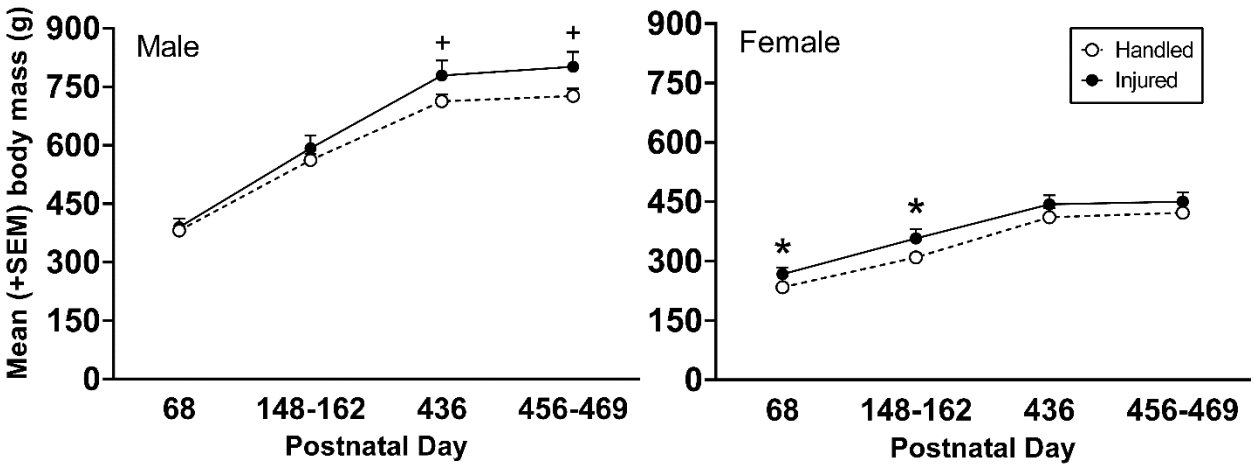

Figure 5.1 The effects of neonatal injury on body mass in rats previously shown to have injury-induced hippocampal-dependent memory deficits (Henderson et al., in press)
In that study, male rats manifested injury-induced memory deficits in middle age whereas female rats were only impaired in adulthood. The present findings show that neonatal injury tended to increase body mass on P436 and on P456-463 in the male rats, but had no effect at earlier ages. In female rats, neonatal injury increased body mass on P68 and P148-162, but not in middle age. $^{*}p < 0.05$, $^{+}p = 0.07$ vs. same-sex handled

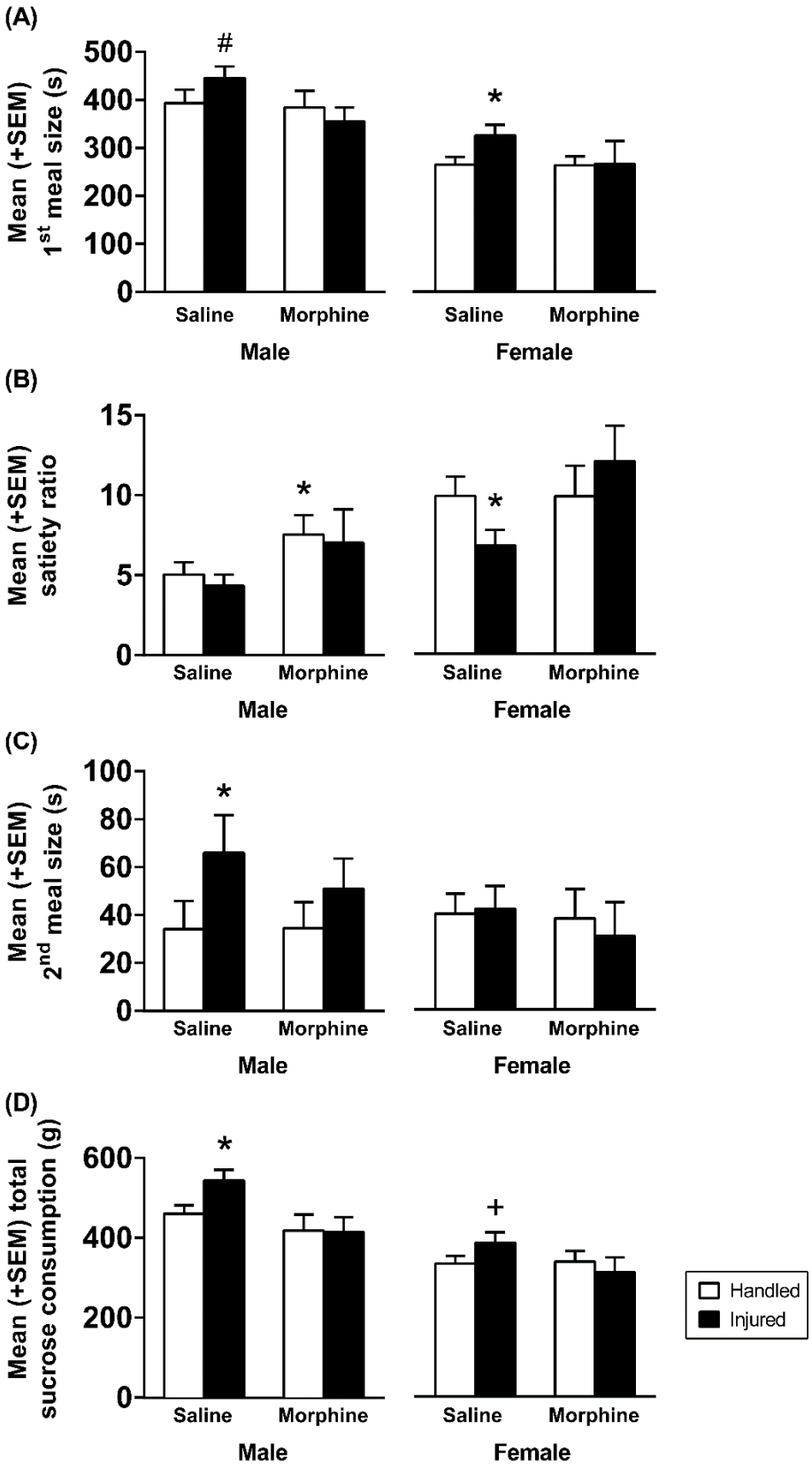

Figure 5.2 The effects of neonatal injury and preemptive morphine treatments on sucrose consumption

In male rats (P231-255), neonatal injury (A) tended to increase the size of the first sucrose meal, (B) increased the size of the second sucrose meal, and (D) increased total sucrose consumption on the experimental day. In female rats (P242), neonatal injury (A) increased the size of the first sucrose meal, (C) decreased the satiety ratio, and (D) tended to increase total sucrose consumption. Morphine treatments at the time of neonatal injury prevented the effects of neonatal injury on adult sucrose consumption. Morphine treatments in the absence of pain (C) increased the satiety ratio in male rats. $^{*}p \leq 0.05$, $^{+}p = 0.06$, $^{\#}p = 0.08$ vs. same-sex handled-saline rats

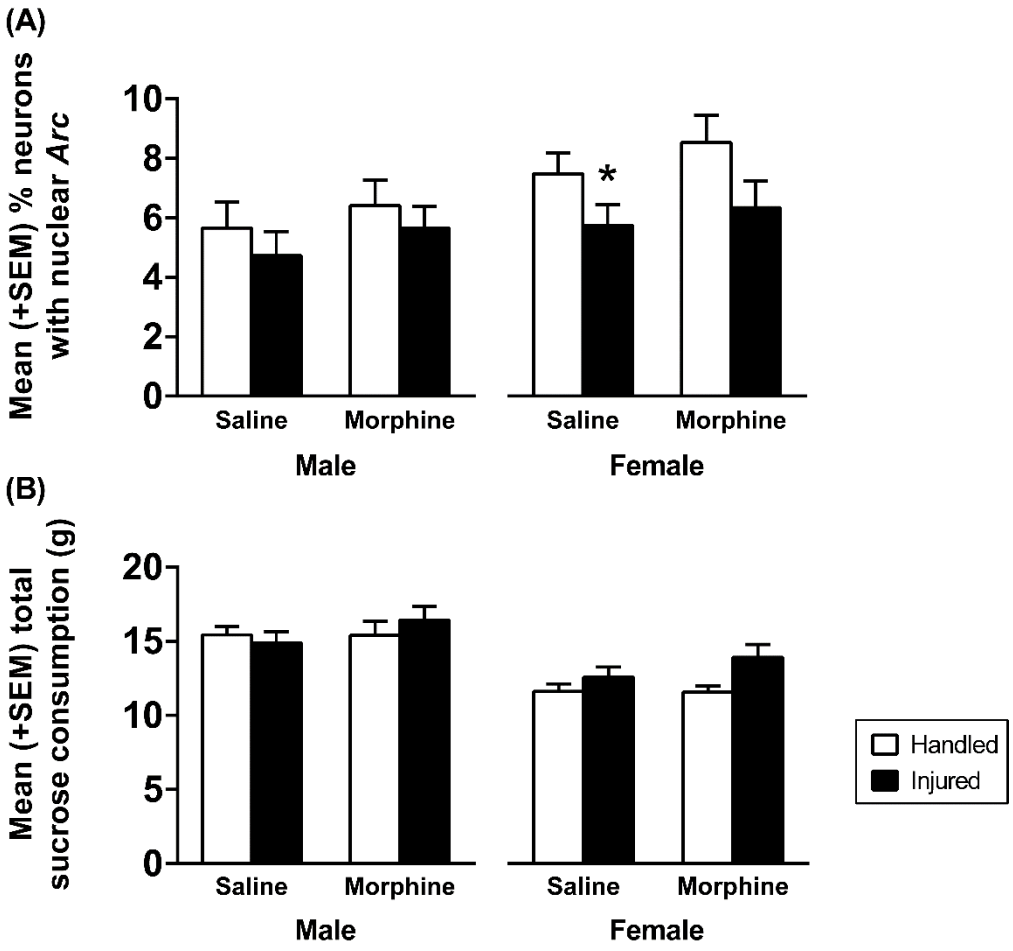

Figure 5.3 The effects of neonatal injury and morphine treatments on sucrose-induced *Arc* mRNA expression in dCA1 neurons

In female rats (P307-401), neonatal injury (A) attenuated sucrose-induced *Arc* expression and morphine treatments at the time of injury effect. These effects of injury on sucrose-induced Arc expression was not due to effects of injury on (B) the amount of sucrose solution consumed during the 7-min period. In male rats (P315-389), neonatal injury and morphine treatments did not affect both of the measures. *$p < 0.05$ vs. same-sex handled-saline rats

6 CHAPTER 6: GENERAL DISCUSSION

The goal of this dissertation was to test our overarching hypothesis that the dorsal hippocampus forms a memory of a meal and inhibits meal onset during the postprandial period (ppIMI). Specifically, this dissertation asked the following questions: (1) Does an eating episode induce synaptic plasticity as measured by *Arc* expression in dorsal hippocampal neurons? (2) Does inactivation of dorsal hippocampal neurons following an eating episode accelerate the onset of the next meal? (3) Are injury-induced chronic hippocampal-dependent memory deficits associated with disrupted meal patterning, attenuated meal-induced synaptic plasticity, and altered body mass? Collectively, the present findings indicate that orosensory sweet stimulation is sufficient to induce synaptic plasticity as measured by *Arc* expression in dorsal hippocampal neurons (Chapter 2) and that this post-meal activity in the dorsal hippocampus inhibits meal onset (Chapter 3). Additionally, a manipulation that produces long-lasting hippocampal-dependent memory deficits (Chapter 4) is associated with disrupted meal patterning, attenuated meal-induced synaptic plasticity in dorsal hippocampal neurons, and increased body mass (Chapter 5). The present dissertation supports a novel model of obesity; hippocampal dysfunction contributes to the development and/or maintenance of diet-induced obesity (Davidson et al., 2005; Davidson et al., 2014; Kanoski & Davidson, 2011; Parent et al., 2014).

6.1 Chapter 2 Findings

The present finding that sucrose consumption increases dCA1 *Arc* expression, a neuronal marker of synaptic plasticity, suggests that dCA1 neurons form a memory of eating episodes (i.e., oral stimuli). This interpretation is likely, given previous studies

demonstrating that *Arc* is critical for long-term memory. Specifically, manipulations that inhibit *Arc* in the hippocampus produce memory deficits (Bramham et al., 2010; Czerniawski et al., 2011; Guzowski et al., 2000; McIntyre et al., 2005; Messaoudi et al., 2007; Plath et al., 2006; Shepherd & Bear, 2011). Moreover, sustained *Arc* translation is required for consolidation of long-term potentiation (LTP; Bramham et al., 2010; Messaoudi et al., 2007), which is a cellular mechanism involved in memory consolidation (Lynch, Rex, & Gall, 2007). In addition, Arc protein contributes to dendritic remodeling, specifically by increasing F-actin in dendritic spines (Bramham et al., 2010; Korb & Finkbeiner, 2011; Messaoudi et al., 2007; Shepherd & Bear, 2011). Dendritic spine growth is critical for the longevity of LTP (Fukazawa et al., 2003; Lynch et al., 2007); therefore, it is possible that eating episodes induce *Arc* activation in dCA1 neurons, which then induces cellular and morphological changes (i.e., structural plasticity) to facilitate long-term storage of the memory of an eating event.

While eating episodes can induce structural plasticity, it is unlikely that all eating episodes become long-term memories. Given the frequency of food intake, retaining long-term memories of eating episodes would cause saturation of synaptic plasticity. So what does meal-induced *Arc* reflect? Morris and colleagues propose a type of episodic memory that results from “automatic recording of attended experience” (Morris, 2001, 2006; Morris & Frey, 1997; Morris et al., 2003). Specifically, they propose that the hippocampus consolidates and stores unanticipated events in real time (Morris, 2006). These memories are stored as episodic memories, but they rapidly decay unless protein synthesis occurs for memory persistence (Morris, 2006). Thus, it is possible that sucrose- and saccharin-induced *Arc* expression reflects the automatic recording of a

memory that fades over time (Morris, 2001, 2006; Morris & Frey, 1997; Morris et al., 2003).

We found that extended training with a sucrose solution attenuated sucrose-induced *Arc* in dCA1 neurons, which suggests that dCA1 neurons are less likely to form a memory of a repeatedly-experienced meal. This interpretation is consistent with previous studies showing that extensive behavioral training in the spatial water maze (Guzowski, Setlow, et al., 2001) and on a lever-pressing task (Kelly & Deadwyler, 2002, 2003), as well as repeated exposure to the same environment within a day (Guzowski et al., 2006), decreases behavior-induced *Arc* expression in hippocampal CA1 neurons. Thus, our findings suggest two things: (1) repeated exposure to sweet orosensory stimuli acts in a similar manner, and can affect the degree of *Arc* expression and (2) increased experience with a particular meal may attenuate the ability of that meal to induce synaptic plasticity as measured by *Arc* expression in dCA1 neurons.

We also found that consumption of a non-caloric sweetener saccharin induces *Arc*. This finding indicates that orosensory stimulation is sufficient to induce synaptic plasticity as measured by *Arc* expression in dCA1 neurons. Unexpectedly, we found that saccharin consumption induced higher *Arc* expression in dCA1 neurons compared to consumption of an isopreferred concentration of sucrose. This finding was surprising given that postingestive consequences directly and/or indirectly activate the hippocampus. For example, gastric distension-sensitive neurons were recently discovered in dCA1 (Xu et al., 2014). Furthermore, the hippocampus expresses moderate to high densities of prandial signal receptors including leptin, insulin, ghrelin, glucose, cholecystokinin, and bombesin-like peptides (Acosta, 2001; Battey & Wada,

1991; Hakansson, Brown, Ghilardi, Skoda, & Meister, 1998; Hollingsworth, 1989; Jennings et al., 2003; Kamichi et al., 2005; Lathe, 2001; Mercer et al., 1996; Sebret et al., 1999; Tirassa, Stenfors, Lundeberg, & Aloe, 1998; Werther et al., 1987; Wolf & Moody, 1985; Zigman, Jones, Lee, Saper, & Elmquist, 2006). Some prandial hormones and peptides, such as leptin and insulin, are permeable through the blood brain barrier (BBB; Banks, Jaspan, Huang, & Kastin, 1997; Banks, Kastin, Huang, Jaspan, & Maness, 1996), and food intake induces the release of prandial bombesin-like peptides peripherally as well as in the hippocampus (Kateb & Merali, 1992; Merali & Kateb, 1993; Merali, McIntosh, & Anisman, 1999). Interestingly, administration of these food-related hormones and peptides enhances hippocampal functioning and morphology. For example, the administration of ghrelin or leptin facilitates neurogenesis in the hippocampal dentate gyrus region (Garza et al., 2008; Moon et al., 2009), and peripheral and intrahippocampal injections of ghrelin increase hippocampal synaptic plasticity and spine density (Carlini et al., 2010; Chen et al., 2011; Diano et al., 2006). Moreover, peripheral and/or intrahippocampal injections of leptin, insulin, ghrelin, or bombesin enhance hippocampal-dependent learning and memory in a variety of memory tasks (Babri et al., 2013; Babri, Badie, Khamenei, & Seyedlar, 2007; Carlini et al., 2007; Carlini et al., 2004; Chen et al., 2011; Craft et al., 1996; Diano et al., 2006; Farr et al., 2006; Gisou et al., 2009; McNay et al., 2010; Moosavi, Naghdi, Maghsoudi, & Zahedi Asl, 2006; Oomura et al., 2006; Roesler et al., 2006; Roesler et al., 2009). Therefore, it is likely that food intake, including sucrose consumption, enhances hippocampal activity during the postprandial period via release of food-related hormones and peptides.

Then how do we explain significant differences in *Arc* levels induced by saccharin versus sucrose? It is unlikely that differences in reinforcing effects affected *Arc* levels, given that 4% sucrose and 0.2% saccharin were preferred equally in these rats (Chapter 3 findings; Messier & White, 1984; J. C. Smith & Sclafani, 2002). Also, the finding that sucrose sham feeding increases extra-cellular dopamine levels in nucleus accumbens in rats suggests that a lack of postingestive consequences does not reduce the rewarding properties of sucrose taste (Hajnal et al., 2004). Rather, it is possible that 7 min of 4% sucrose consumption was not sufficient to induce as much *Arc* activation as 0.2% saccharin consumption due to lack of satiety from the sucrose solution. For example, saccharin produces oral satiety (Mook et al., 1980), which is a state that terminates further intake without any significant postingestive effects including gastrointestinal distention (Kushner & Mook, 1984; Mook et al., 1980; Sclafani & Nissenbaum, 1985), while other solutions with caloric contents (e.g., glucose solutions) do not (Sclafani & Nissenbaum, 1985). Thus, it is possible that lack of oral satiety from sucrose solution could have contributed to the difference.

6.2 Chapter 3 Findings

We showed that temporary inactivation of dorsal hippocampal neurons immediately following an eating bout accelerates meal onset and increases the size of the next meal. The effects of inactivation through muscimol were specific, in that muscimol did not affect licking speed. The present findings are consistent with previous studies showing that permanent fornix or hippocampal lesions increase food intake (Davidson et al., 2005; Forloni, Fisone, Guaitani, Ladinsky, & Consolo, 1986; King, Arceneaux, Cook, Benjamin, & Alheid, 1996) and meal frequency (Clifton et al., 1998;

Davidson & Jarrard, 1993; Davidson et al., 2005), and decrease the ppIMI (Osborne & Dodek, 1986). Unlike permanent hippocampal lesions, however, temporary inactivating the dorsal hippocampal neurons allowed us to observe the effects of hippocampal inactivity during the ppIMI without affecting the size of the preceding meal. To be more specific, permanent hippocampal lesions could affect the duration of ppIMI by also affecting the size of the preceding meal. Thus, our study shows that activity of dorsal hippocampal neurons following a meal is sufficient or necessary to inhibit meal onset.

Intrahippocampal muscimol infusions were specifically timed to occur during a critical period for memory formation and consolidation (i.e., following acquisition) in our study. Based on the findings from Chapter 2, it is highly likely that hippocampal inactivation occurred during the time window of sucrose-induced *Arc* transcription. Therefore, the present findings suggest that meal memory formation may be necessary for inhibiting meal onset. This interpretation is consistent with clinical observations that hippocampal-dependent memory deficits are associated with overeating. For example, patients with temporal lobe lesions including the hippocampus cannot remember an eating episode and have shorter ppIMIs (Hebben et al., 1985; Higgs et al., 2008; Rozin et al., 1998). In addition, eating in distracting environments (e.g., watching television) impairs memory of the eating episode and causes participants to consume more food at the next bout (Higgs & Woodward, 2009; Oldham-Cooper et al., 2011). In contrast, recall of the most recent meal suppresses subsequent food intake relative to recall of a meal that was consumed the previous day or of recent non-food related events (Higgs, 2002). Furthermore, television viewing is not only associated with increased total intake, but also with increased meal frequency and decreased ppIMI (Stroebele & de Castro,

2004). However, the present findings do not rule out the possibility that intrahippocampal muscimol infusions accelerated meal onset by influencing other processes in addition to memory. For example, inactivation of the dorsal hippocampus may have inhibited the effects of postingestive satiety signals, and specifically those produced by gastric distention (Xu et al., 2014). Moreover, hippocampal inactivation may have accelerated meal onset by interfering with the ability of hippocampal neurons to keep track of elapsed time (Deshmukh & Bhalla, 2003; Itskov et al., 2011; MacDonald et al., 2011; Sinden et al., 1986; Young & McNaughton, 2000).

6.3 Chapter 4 and 5 Findings

Our findings indicate that neonatal injury impaired hippocampal-dependent memory and disrupted meal patterning. Importantly, our results showed that memory deficits were associated with disrupted energy homeostasis. For example, the timing of the differences in memory deficits observed between male and female rats paralleled the timing of their increases in body mass. Moreover, our findings showed that morphine administration at the time of injury eliminated these injury-induced deficits, indicating that these injury-induced deficits are preventable.

What common hippocampal mechanism(s) has contributed to both injury-induced memory deficits and disrupted energy homeostasis? Painful experiences are associated with stress; therefore, it is possible that injury-induced stress and not injury during the neonatal period contributed to hippocampal-dependent memory deficits in neonatally-injured rats. Plasma corticosterone (CORT) levels are significantly elevated in neonatally-injured rat pups for at least 1-week post-injury (Victoria, Karom, Eichenbaum, et al., 2014). Moreover, stress during the neonatal period has been shown to impact

adult hippocampal structure and function negatively (Brunson et al., 2001; Cui et al., 2006; Maras & Baram, 2012; Oomen et al., 2010). For example, stress induced by poor maternal care elevates CORT release in rat pups and impairs hippocampal-dependent memory in middle-aged rats (Brunson et al., 2005).

Clinically, preterm infants are more likely to gain weight and/or develop obesity-related complications such as metabolic syndrome later in life (Dulloo et al., 2006; Euser et al., 2005; Fagerberg et al., 2004; Finken et al., 2006; Hofman et al., 2004; Singhal et al., 2003; Uthaya et al., 2005; Yeung, 2006). Furthermore, experiencing physical and/or psychological stressors during early stages of life increases adipose tissue mass and induces hyperinsulinemia in rats (Haley et al., 2013) and in humans, it is associated with obesity and metabolic syndrome in adulthood (D'Argenio et al., 2009; Gunstad et al., 2006; Rohde et al., 2008; Vanitallie, 2002).

Our present and previous findings indicate that neonatal inflammatory pain-induced deficits are preventable. Specifically, we showed that morphine administration at the time of injury eliminated both the injury-induced memory deficits and the disrupted meal patterning. These positive effects of morphine were also observed in previous studies showing that morphine treatments prevented behavioral changes in responses of adults to pain- and anxiety-provoking stimuli (LaPrairie et al., 2008; Victoria, Karom, & Murphy, 2014). Our morphine findings, however, do not reveal whether pain, pain-related stress, inflammation, or a combination of these factors are necessary to produce these deficits. For example, morphine alleviates injury-induced pain, and also decreases injury-induced cytokines including IL-1β (Clark et al., 2007). Given that central administration of cytokines impairs memory (Barrientos et al., 2002; Gonzalez et

al., 2009; Goshen et al., 2007; Machado et al., 2010; Oitzl et al., 1993; Pugh et al., 1999) and hippocampal IL-1β is significantly increased in genetically-modified obese mice (Erion et al., 2014), it is possible that injury-induced cytokine release contributed to the observed deficits in spatial memory and energy homeostasis.

6.4 Summary

In sum, our experiments led to several novel findings. First, the results of Chapter 2 suggest that dorsal hippocampal neurons form a memory of a meal, and also that the taste of sweetness without postingestive consequences is sufficient to induce *Arc* expression in dCA1 neurons. Interestingly, our findings also indicate that increasing the amount of experience with a meal alters the ability of the dorsal hippocampal neurons to form a memory of that meal, such that extensive training with sucrose attenuated sucrose-induced *Arc* expression in dCA1 neurons. Second, findings from Chapter 3 showed that dorsal hippocampal neural activity during the postprandial period inhibits energy intake. Given that we specifically inhibited activity of dorsal hippocampal neurons following sucrose intake, possibly during the same time period when *Arc* expression is also being transcribed, this finding suggests that postprandial hippocampal *Arc* expression may be necessary to inhibit meal onset. Findings from Chapters 4 and 5 demonstrated that neonatal injury impairs hippocampal-dependent memory, and that these memory deficits are associated with increased energy intake and body mass. Although we did not show that hippocampal-dependent memory deficits are sufficient to disrupt energy homeostasis, our findings that morphine treatments at the time of injury prevented injury-induced memory deficits and disrupted meal

patterning indicate that experiencing inflammatory pain on the day of birth produces detrimental and long-lasting deficits.

6.5 Why is this important?

Davidson and colleagues propose that diet-induced obesity is caused and maintained by a vicious cycle wherein excess intake disrupts hippocampal function, which further increases intake (Davidson et al., 2005; Davidson et al., 2014; Kanoski & Davidson, 2011). Our finding that inhibiting dorsal hippocampal neurons with muscimol accelerated meal onset and increased meal size supports their hypothesis that memory deficits can precede the development of obesity (Davidson et al., 2005; Davidson et al., 2014; Kanoski & Davidson, 2011). The present results do not reveal which came first: the memory deficits or the weight gain. However, our findings that (1) eating episodes induce hippocampal synaptic plasticity, and (2) hippocampal-dependent memory deficits are associated with disrupted homeostasis provide new insight into the involvement of hippocampal dysfunction, and specifically hippocampal-dependent memory, may be related to diet-induced obesity (Parent et al., 2014). Given that the prevalence of obesity remains high (Ogden et al., 2014), findings from the present dissertation could lead to more effective and evidence-based treatments for obesity.

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other regions involved in brain reward circuitry. *Proc Natl Acad Sci U S A, 103*(42), 15641-15645. doi: 10.1073/pnas.0601977103

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Weingarten, H. P. (1984). Meal initiation controlled by learned cues: basic behavioral properties. *Appetite, 5*(2), 147-158.

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Wobbrock, J. O., Findlater, L., Gergle, D., & Higgins, J. J. (2011, May 7–12, 2011). *The aligned rank transform for nonparametric factorial analyses using only anova procedures.* Paper presented at the Annual CHI Conference on Human Factors in Computing Systems, Vancouver, BC, Canada.

Wolf, S. S., & Moody, T. W. (1985). Receptors for GRP/bombesin-like peptides in the rat forebrain. *Peptides, 6 Suppl 1*, 111-114.

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Wright, R. L., Lightner, E. N., Harman, J. S., Meijer, O. C., & Conrad, C. D. (2006). Attenuating corticosterone levels on the day of memory assessment prevents chronic stress-induced impairments in spatial memory. *Eur J Neurosci, 24*(2), 595-605. doi: 10.1111/j.1460-9568.2006.04948.x

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related gene expression in inflamed tissue. *J Neurochem, 103*(4), 1628-1643. doi: 10.1111/j.1471-4159.2007.04874.x

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Zigman, J. M., Jones, J. E., Lee, C. E., Saper, C. B., & Elmquist, J. K. (2006). Expression of ghrelin receptor mRNA in the rat and the mouse brain. *J Comp Neurol, 494*(3), 528-548. doi: 10.1002/cne.20823

Zorrilla, E. P., Inoue, K., Fekete, E. M., Tabarin, A., Valdez, G. R., & Koob, G. F. (2005). Measuring meals: structure of prandial food and water intake of rats. *Am J*

Physiol Regul Integr Comp Physiol, 288(6), R1450-1467. doi:

10.1152/ajpregu.00175.2004

APPENDICES

Appendix A Curriculum Vitae

Yoko Ogawa Henderson

Neuroscience Institute

Georgia State University

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Atlanta, GA 30303

(404) 413-6345

yogawa1@student.gsu.edu

EDUCATION:

DEGREE: Bachelor of Science

MAJOR: Psychology

INSTITUTION: Wittenberg University, Springfield, OH (Fall 2001-Spring 2005)

DEGREE: Master of Arts

MAJOR: General Psychology

INSTITUTION: East Tennessee State University, Johnson City, TN (Fall 2005-Summer 2007)

DEGREE: Master of Science

MAJOR: Neuroscience

INSTITUTION: Georgia State University, Atlanta, GA (Fall 2007-Summer 2012)

DEGREE: Doctor of Philosopy

MAJOR: Neuroscience

INSTITUTION: Georgia State University, Atlanta, GA (Fall 2012- Spring 2015)

RESEARCH EXPERIENCE:

Summer & Spring 2004, Undergraduate Research Assistant, Advisor: Dr. Josephine F. Wilson, Wittenberg University, Springfield, OH

Summer 2006, Office of Continuing Medical Education Intern, Advisor: Barbara Sucher, MBA (Associate Dean for CME), East Tennessee State University, James H. Quillen College of Medicine, Johnson City, TN

Fall 2005-Spring 2007, Graduate Student, Advisor: Dr. Russ W. Brown, East Tennessee State University, Johnson City, TN

Summer 2007, Research Assistant, Advisor: Dr. Marise B. Parent, Georgia State University, Atlanta, GA

Fall 2007-present, Graduate Student, Advisor: Dr. Marise B. Parent, Georgia State University, Atlanta, GA

PEER-REVIEWED PUBLICATIONS:

Henderson, Y. O., Victoria, N. C., Inoue, K., Murphy, A. Z., & Parent, M. B. (in press). Early life inflammatory pain induces long-lasting deficits in hippocampal-

dependent spatial memory in male and female rats. *Neurobiol Learn Mem*, *118C*, 30-41. doi: 10.1016/j.nlm.2014.10.010

Parent, M. B., Darling, J. N., & **Henderson, Y. O.** (2014). Remembering to eat: hippocampal regulation of meal onset. *Am J Physiol Regul Integr Comp Physiol*, *306*(10), R701-713. doi: 10.1152/ajpregu.00496.2013

Henderson, Y. O., Smith, G. P., & Parent, M. B. (2013). Hippocampal neurons inhibit meal onset. *Hippocampus*, *23*(1), 100-107. doi: 10.1002/hipo.22062 ***Faculty of 1000 Prime Recommended**

Perna, M. K., **Henderson, Y. O.**, Bruner, C. L., & Brown, R. W. (2011). An analysis of nicotine conditioned place conditioning in early postweanling and adolescent rats neonatally treated with quinpirole. *Behav Brain Res*, *220*(1), 254-261. doi: 10.1016/j.bbr.2011.02.004

POSTERS AND PRESENTATIONS:

Wilson, J. F., **Ogawa, Y.**, & Engle, K. Increased blood sugar levels associated with food variety. Poster presented at the Annual Meeting of the Society for the Study of Ingestive Behavior, Pittsburgh, PA, 2005.

Ogawa, Y., Brown, R. W., Woodruff, M. L., & Yin, D. Cognitive deficits and genetic alterations produced by chronic stress in mice. Poster presented at the Annual Appalachian Student Research Forum, Johnson City, TN, 2006. ***Highlighted in *Johnson City Press***

Maple, A. M., Perna, M. K, **Ogawa, Y.**, Longacre, I. D., Woodruff, M. L., & Brown, R. W. Nicotine alleviation of deficits in prepulse inhibition in a rodent model of

schizophrenia are blocked by mecamylamine. Poster presented at the College on Problems of Drug Dependence Annual Meeting, Scottsdale, AZ, 2006.

Baisden, R. H., **Ogawa, Y.**, Cooper, E. L., Bruner, C. L., Perna, M. K., Thompson, K. N., & Brown, R. W. The effects of nicotine conditioned place preference in D_2-primed adolescent rats: Age-related and gender effects. Poster presented at the Society for Neuroscience Annual Meeting, Atlanta, GA, 2006.

Abraham, J., **Ogawa, Y.**, Cooper, E. L., & Brown R. W. The effects of nicotine conditioned place preference in D_2-primed adolescent rats: Age-related and gender effects. Poster presented at the Annual Appalachian Student Research Forum, Johnson City, TN, 2007.

Fridie, L. C., **Ogawa, Y.**, & Parent, M. B. Eating a meal increases *Arc* mRNA expressions in hippocampal neurons. Poster presented at the Behavioral Research Advancements in Neuroscience Summer Symposium, Atlanta, GA, 2010.

Galloway, C. R., **Ogawa, Y.**, & Parent, M. B. The role of the hippocampus in influencing meal onset. Poster presented at the Psychology Undergraduate Research Conference, Atlanta, GA, 2010.

Ampudia, J. A., **Ogawa, Y.**, & Parent, M. B. The effects of again in female rats on inter-meal intervals. Poster presented at the Louis Stokes Alliances for Minority Participation Conference, Atlanta, GA, 2010.

Filitor R. W. A, Galloway, C. R., **Ogawa, Y.**, & Parent, M. B. Neonatal injury may impair hippocampal-dependent memory in middle-aged rats. Poster presented at the

Georgia Undergraduate Research in Psychology Conference, Kennesaw, GA, 2011.

Ogawa, Y. & Parent, M. B. The hippocampus formulates a memory of a meal and instantiates inter-meal interval. Poster presented at the Brains & Behavior Annual Spring Retreat, Atlanta, GA, 2011.

Galloway, C. R., **Ogawa, Y.**, & Parent, M. B. The hippocampus regulates meal timing. Poster presented at the Georgia Undergraduate Research in Psychology Conference, Kennesaw, GA 2011. ***Outstanding Poster Presentation: 1st place**

Galloway, C. R., **Ogawa, Y.**, & Parent, M. B. The hippocampus regulates meal timing. Poster presented at the Georgia State Undergraduate Research Conference, Atlanta, GA, 2011.

Galloway, C. R., **Ogawa, Y.**, & Parent, M. B. The hippocampus regulates meal timing. Poster presented at the Colonial Academic Alliance Undergraduate Research Conference, Hempstead, NY, 2011.

Jimenez, A. N., **Ogawa, Y.**, & Parent, M. B. Eating a meal increases *Arc* mRNA expressions in the dorsal hippocampus of Sprague-Dawley rats. Poster presented at the MBRS-RISE 2011 Summer Research Presentations, Atlanta, GA, 2011

Ogawa, Y., Smith, G. P., & Parent, M. B. To eat or not to eat: Hippocampal involvement in meal onset. Poster presented at the Society for Neuroscience Annual Meeting, Washington, DC, 2011.

Parent, M. B., **Ogawa, Y.**, Victoria, N. C., & Murphy, A. Z. Impact of neonatal pain and inflammation on hippocampal-dependent memory in middle-aged rats. Poster presented at the Society for Neuroscience Annual Meeting, Washington, DC, 2011.

Parent, M. B., **Ogawa, Y.**, Victoria, N. C., & Murphy, A. Z. Neonatal pain and inflammation impairs hippocampal-dependent memory in an age-dependent manner in male rats. Oral presentation at the XXXVI Winter Conference on the Neurobiology of Learning & Memory, Park City, UT, 2012.

Ogawa, Y., Smith, G. P., & Parent, M. B. Hippocampal neurons inhibit meal onset. Oral presentation at the XXXVI Winter Conference on the Neurobiology of Learning & Memory, Park City, UT, 2012.

Ogawa, Y. To eat or not to eat: Hippocampal neurons inhibit meal onset. Oral presentation at the Annual Animal Behavior Conference, Bloomington, IN, 2012.

Parent, M. B., **Ogawa, Y.**, Victoria, N. C., & Murphy, A. Z. Impact of neonatal pain and inflammation on hippocampal-dependent memory in middle-aged rats. Poster presented at the Brains & Behavior Annual Spring Retreat, Atlanta, GA, 2012.

Ogawa, Y., Victoria, N. C., Murphy, A. Z., & Parent, M. B. Neonatal inflammatory pain increases food intake and body weight in adulthood and impairs hippocampal-dependent memory. Poster presented at the Society for Neuroscience Annual Meeting, New Orleans, LA, 2012.

Parent, M. B., **Ogawa, Y.**, Victoria, N. C., & Murphy, A. Z. Interrelated effects of neonatal inflammatory pain on memory, meal onset, and body mass. Oral

presentation at the XXXVII Winter Conference on the Neurobiology of Learning and Memory, Park City, UT, 2013.

Schmuck, L. M., **Henderson, Y. O.**, Victoria, N. C., Murphy, A. Z., & Parent, M. B. Neonatal pain accelerates meal onset and increases body mass in adult female rats with poor spatial memory. Poster presented at the Georgia State Undergraduate Research Conference, Atlanta, GA, 2013.

Nagar, N., **Henderson, Y. O.**, Victoria, N. C., Murphy, A. Z., & Parent, M. B. Neonatal pain and inflammation impairs hippocampal-dependent memory in middle-aged rats. Poster presented at the Georgia State Undergraduate Research Conference, Atlanta, GA, 2013.

Henderson, Y. O. To eat or not to eat: Contributions of dorsal hippocampal neurons and memory to meal onset. Oral presentation at the Brains & Behavior Annual Spring Retreat, Atlanta, GA, 2013.

Henderson, Y. O., Murphy, A. Z., & Parent, M. B. Neonatal inflammatory pain-induced increases in food intake and body mass in adulthood are associated with hippocampal-dependent memory deficits. Poster presented at the Society for Behavioral Neuroendocirnology Annual Meeting, Atlanta, GA, 2013.

Gentry, J. N., **Henderson, Y. O.**, & Parent, M. B. Interrelated effects of neonatal injury on meal intake and hippocampal-dependent memory. Poster presented at the NET/work Symposium, Atlanta, GA, 2013.

Gentry, J. N., **Henderson, Y. O.** & Parent, M. B. Interrelated effects of neonatal injury on meal intake and hippocampal-dependent memory. Poster presented at the BRAIN/B&B/SEED Research Symposium, Atlanta, GA, 2013.

Gentry, J. N., **Henderson, Y. O.** & Parent, M. B. Interrelated effects of neonatal injury on meal intake and hippocampal-dependent memory. Poster presented at the SAEOPP McNair/SSS Scholars Research Conference, Atlanta, GA, 2013.

Parent, M. B., **Henderson, Y. O.**, Victoria, N. C., & Murphy, A. Z. Preemptive morphine analgesia prevents the impairing effects of neonatal inflammatory pain on adult hippocampal dependent memory and produces memory deficits in non-injured rats. Poster presented at the Society for Neuroscience Annual Meeting, San Diego, CA, 2013.

Henderson, Y. O., Vazdarjanova, A., Murphy, A. Z., & Parent, M. B. Eating-associated hippocampal expression of the synaptic plasticity marker *Arc* correlates with the duration of the postprandial intermeal interval and is diminished in rats with poor hippocampal-dependent spatial memory. Poster presented at the 31st Annual Scientific Meeting of the Obesity Society, Atlanta, GA, 2013.

Henderson, Y. O., Vazdarjanova, A., Murphy, A. Z., & Parent, M. B. Eating-associated hippocampal expression of the synaptic plasticity marker *Arc* correlates with the duration of the postprandial intermeal interval and is diminished in rats with poor hippocampal-dependent spatial memory. Poster presented at the Brains & Behavior Spring Annual Retreat, Atlanta, GA, 2014.

Parent, M. B., **Henderson, Y. O.**, & Vazdarjanova, A. Sparse encoding of a memory of a meal in the dorsal hippocampus as revealed with Arc expression. Poster presented at the Society for Neuroscience Annual Meeting, Washington, DC, 2014.

PROFESSIONAL SOCIETIES:

Psi Chi, Fall 2006-present

Center for Behavioral Neuroscience, Fall 2007-present

Society for Neuroscience, 2011-2012

Excellence in Science Program, American Association for the Advancement of Science, Summer 2012-present

HONORS, AWARDS and FELLOWSHIPS:

Graduate Assistantship/Tuition Scholar, Department of Psychology, East Tennessee State University, Fall 2005-Spring 2007

Travel funds for the Annual Animal Behavior Conference at Indiana University, Center for Behavioral Neuroscience/Emory Neuroscience Initiative, Spring 2012

Dissertation Award, Georgia State University, Spring, 2012

Alumnae Educational Grant, Chi Omega Foundation, Fall 2012

Brains & Behavior Program Fellowship, Georgia State University, Fall 2012-Present

TEACHING EXPERIENCE:

Japanese tutor, East Asian Studies Department, Wittenberg University, Springfield, OH, Fall 2001-Spring 2002

Instructor, PSYC 3030 Principles and Methods of Psychological Investigation, Georgia State University, Atlanta, GA, Spring 2009

Instructor, PSYC 3030 Principles and Methods of Psychological Investigation, Georgia State University, Atlanta, GA, Fall 2009

Instructor, PSYC 3530 Advanced Research Design and Analysis, Georgia State University Atlanta, GA, Spring 2010

Tutor, Psychology Department Writing Center, Georgia State University, Atlanta, GA, Fall 2010

Instructor, Improving Motivation, Performance and Attitudes of Children and Teachers University of West Georgia, Carrolton, GA, October 2011

Brain Awareness Campaign educator at Columbia Middle School, Decatur, GA, The Atlanta Chapter of the Society for Neuroscience, April 2012

Substitute lecturer (1 class), NEUR 4200 Neurobiology of learning and memory, Georgia State University, Atlanta, GA, March 2013

Substitute lecturer (1 class), NEUR 4200 Neurobiology of learning and memory, Georgia State University Atlanta, GA, November 2013

TECHNIQUES, SKILLS and BEHAVIORAL ASSAYS:

Spatial water maze

Inhibitory avoidance

Open-field

Spontaneous alternation

Conditioned place preference

Elevated T-maze

Marble burying

Object recognition

Perfusion

Stereotactic surgery

Cryostat brain sectioning (for Nisl, autorad, and catFISH)

Nissl thionin staining

Injections (intracranial, intramuscular, intraperitoneal, and subcutaneous)

Stereological cell counting and classification with Zeiss LSM Image Browser

Rodent euthanasia (postmortem tissue collection)

Rodent brain dissection

Enzyme-linked immunosorbent assay

Lowry DC protein assay

Rodent estrous cycle determination

Densitometry with Scion Image Software

SERVICE:

Peace Corps volunteer in Morocco, Leysin American School, Switzerland, March 2001

Volunteer, The Bridge House, Springfield, OH, Fall 2002

Secretary, American International Association, Wittenberg University, Springfield, OH, Fall 2002-Spring 2004

Volunteer, Make a Wish Foundation, Ohio, Kentuckey and Indiana Chapter, Columbus, OH, Fall 2003-Spring 2005

Volunteer, Department of Literature and Language, East Tennessee State University, Johnson City, TN, Fall 2005-Spring 2007

International Ambassador, East Tennessee State University, Johnson City, TN, Spring 2007

Participated in 5K India Run for Hope, American Cancer Society India Cancer Initiative, Atlanta, GA, August 2007

Undergraduate Placement program coordinator, Neuroscience Graduate Student Association, Georgia State University, Atlanta, GA, Fall 2011

Participated in Walk to End Alzheimer's, Alzheimer's Association, Atlanta, GA, September 2011

Co-President, Neuroscience Graduate Student Association, Georgia State University, Atlanta, GA, Fall 2011-Spring 2012

STUDENTS MENTORED:

Anthony Iacono, Georgia State University, Spring 2008-Summer 2009

Ronald Downs, Georgia State University, Summer 2009

Sonum Patel, Georgia State University, Fall 2009-Spring 2010

Kayla Brookshire, Georgia State University, Fall 2009-Spring 2010

Loryn Fridie, Bowdoin College, Summer 2010

Jenine Ampudia, Georgia State University, Summer 2010

Claire Galloway, Georgia State University, Summer 2010-Spring 2011

Richard Austin Filitor, Georgia State University, Spring 2011

Aquila Jimenez, Spelman College, Summer 2011

Amanda Arnold, Georgia State University, Summer 2011

Nivedita Nagar, Georgia State University, Summer 2011-Fall 2013

Geena (Jin A.) Kim, Georgia State University, Fall 2011-Spring 2012

Sandra Chumba, Georgia State University, Fall 2011-Summer 2012

Yasmine Blanch, Georgia State University, Summer 2012-Fall 2012

Lauren Schmuck, Georgia State University, Summer 2012-Spring 2013

Andrew Brown, Georgia State University, Summer 2014-Fall 2014

Sepehr Goshayeshi, Georgia State University, Summer 2014-Fall 2014